VOLUME LVIII NUMBER 2

THE
BOTANICAL GAZETTE

AUGUST 1914

A STUDY OF THE GERMINATING POWER OF SEEDS

MARVIN L. DARSIE, CHARLOTTE ELLIOTT, AND
GEORGE J. PEIRCE

(WITH EIGHTEEN FIGURES)

Scattered throughout botanical literature are many statements regarding the length of time during which seeds may retain their germinating power or, as it is called, their viability. Some of these statements record the germination of seeds from old herbaria, others of seeds supposedly long buried in the earth, and still others of seeds which have been stored for known periods and under known conditions. Few of these records, however, bear critical examination.

Perhaps the earliest authentic records of tests of the continued vitality of air-dry seeds are those of ALPHONSE DE CANDOLLE.[1] In 1832 he first conceived the idea of testing seeds of different species which he had obtained in the harvest of 1831. He kept them all air-dry until May 1846, when he planted 20 seeds of each species. There were 368 species, representing 53 families. Of these but 5 out of 10 species of Malvaceae, 9 out of 45 species of Leguminosae, and 1 out of 30 species of Labiatae showed any power of germination.

Tests of the continued viability of buried seeds were made by DUVEL.[2] He used 109 species of 84 genera and 34 families, and,

[1] DE CANDOLLE, A., Sur la durée relative de la faculté de germer dans des graines appartenant à diverses familles. (Première expérience.) Ann. Sci. Nat. Bot. III. 6:373. 1846.

[2] DUVEL, J. W. T., The vitality of buried seeds. Bull. 83, Bur. Pl. Industry, U.S. Dept. Agric. 1905.

with the exception of two of his duplicate samples, they were all of the harvest of 1902. He mixed his seeds, in definite numbers, with dry clay soil in ordinary flower pots, and buried these in December 1902, to different depths, carefully covering each pot with an earthenware saucer to prevent the loss of seed and other accidents. The majority of the grains of wheat buried 6–8 inches and 36–42 inches had germinated and then decayed, while those at the medium depth of 18–22 inches were merely decayed, without indications of germination, when the pots were taken up in November 1903, eleven months after burial. Approximately all the barley at these three depths had germinated and afterward decayed during the same lapse of time. The majority of the commonly cultivated plants of field and garden could not withstand one year of burial, under the conditions which prevail in the soil outside of Washington, D.C. Many weed seeds, however, showed little deterioration within this length of time.

In 1907 BECQUEREL[3] reported the results of his examination into the germinating power of about 500 species of seeds, belonging to 30 of the more important families of the monocotyledons and dicotyledons, and varying in age from 25 to 135 years. These seeds came from the Muséum d'Histoire Naturelle in Paris. He used 10 seeds of each species, breaking off parts of the integuments of those which seemed impermeable, and, after washing them carefully in distilled water, placed them on damp cotton in crystallizing dishes and kept them at a temperature of 28° C. for more than a month. He obtained germinations in 50 species, all of which were included in four families, namely, the Leguminosae, Nelumbiaceae, Labiatae, and Malvaceae. The oldest seeds which germinated were 3 out of 10 of *Cassia bicapsularis*, one of the Leguminosae, which dated from 1819, and were therefore more than 85 years old at the time of the experiment.

In 1908 EWART[4] published the results of a similar set of tests of over 1000 species of seeds which he found locked in a cupboard in the botanical laboratory at Melbourne, Australia. They had been sent

[3] BECQUEREL, P., Recherches sur la vie latente des graines. Ann. Sci. Nat. Bot. IX. 5:193–311. 1907.

[4] EWART, A. J., On the longevity of seeds. Proc. Roy. Soc. Victoria 21:1–210. 1908.

from Kew in 1856 for the University Gardens, but these not being ready at the time expected, the seeds had been put away in a dark dry closet and had remained there unopened for upward of 50 years. In addition, he examined some ten-year-old seeds from Sydney and Adelaide, and more from the National Herbarium, making altogether nearly 3000 tests. He first tried germinating the seeds in soil, but finding this unsatisfactory, he soaked the seeds and then placed them on moist filter paper in glass dishes and set them in a germinator. Seeds which did not swell after one or two days in water were either filed or treated for 15–90 minutes with concentrated sulphuric acid, that is, until the cuticle was dissolved away. *Adansonia*, for example, required almost 6 hours' treatment of this sort. The seeds were then washed and thereupon swelled readily. EWART adds to his own long list by including some of the results of BECQUEREL, NOBBE, DE CANDOLLE, GIRARDIN, DARWIN, DUVEL, ROMANES, PETER, BERKELEY, and others, making a list, therefore, which comprises about 4000 species. He too found that the majority of those seeds which retain their germinating power for the longest term are members of the Leguminosae, and are generally hard-shelled.

As to the physical and chemical conditions prevailing in dormant seeds there is a diversity of opinion corresponding to the paucity of knowledge. EWART, for example, states that molecular changes and rearrangements continue until finally the seeds no longer retain the power to resume active life. Whatever these molecular changes may be called, whether respiratory or other, it is obviously important, for both theoretical and practical reasons, to determine the conditions in the seeds themselves and the means of maintaining and bettering these if possible. In this paper, however, we are concerned with the results of these conditions rather than with the conditions themselves.

Until recently, so far as we know, tests of the longevity or viability of seeds have depended upon the percentages of actual germinations in prepared beds. Such tests are simple enough in the case of seeds which germinate quickly, and in these cannot be improved upon. When, however, two weeks or more must elapse, even under the most favorable conditions, before one may know the

quality, that is, the germinating power, of the seeds in which one may be interested, a quicker method is desirable for every reason. Not only is the economy of time desirable, possibly for pecuniary reasons, as in the case of a seed-buyer, but also there is less danger, in briefer exposure, of injury or loss from fungous or other enemies of the seeds under examination. One of us has shown that, by using silvered Dewar flasks as calorimeters, one may quickly determine that heat is liberated in the germination of seeds, and has suggested that there may be such differences in the heats liberated by seeds of different ages that one may use these as indicators of age and germinating power or viability.[5] The following experiments were begun in order to test this idea, and they were continued with other and older seeds in order to prove its correctness.

The material used in our experiments came to us through the courtesy of Professor R. A. MOORE, of the University of Wisconsin, Professor E. J. WICKSON, of the University of California, and Professor L. H. PAMMELL, of the Agricultural College at Ames, Iowa, whom we take this opportunity of thanking for their prompt and generous response to our request for seeds of known and considerable age.

Method

The method has been described before. Some of the details, however, as applied to this particular investigation, should be described now. We used silvered Dewar flasks, some made by Burger of Berlin, others not so good, of about 250 cc. capacity. Most of the flasks were round-bottomed, but a few contained a small drainage tube opening into the bottom of the flask. There are differences in the efficiencies of the different flasks even of the same good make and pattern, but, as will appear later, there are great differences between good and bad flasks as insulators. These differences can be ascertained, without destroying the vacuum of the flask, only by using them under constant temperature.

We were fortunate enough to have a convenient constant temperature chamber. This has been sufficiently described before.[6] A maximum-minimum thermometer was taken into the room, but

[5] PEIRCE, G. J., A new respiration calorimeter. BOT. GAZ. **46**:193–202. 1908; also The liberation of heat in respiration. BOT. GAZ. **53**:89–112. 1912.

[6] See PEIRCE, BOT. GAZ. **53**:90, 91. 1912.

the variations in the temperature were so slight that it was not thought to be worth while to continue to record the readings. The slight variations in room temperature shown in the record given below are due mainly to the opening of the room, the presence of one or more of the experimenters, and the heat liberated by a 32 candle-power incandescent light bulb. The light was turned on only as needed, but it is obvious that if the temperature of the room is moderately low, as it was, the heat liberated from a carbon filament lamp of considerable candle-power and radiated and exhaled from the body of an adult individual of average stature, during the 10–40 minutes required for work or observation, would be considerable, and in a smaller room would make a noticeable change in the temperature. In our case the fluctuations were slight.

The thermometers were of two sorts, short and long, the one requiring to be pulled part way out of the flasks to be read, the other long enough to make this unnecessary. Both read to 0°.1 C. The differences in the thermometers, flasks, and cotton plugs used to close the flasks and to hold the thermometers steadily in the necks, as well as the inevitable differences among the seeds themselves, are responsible for such lack as there may be of uniformity in the corresponding results.

The flasks were sterilized by being washed in corrosive sublimate solutions, generally saturated aqueous, and then thoroughly washed out with boiled and cooled distilled water. In many cases the seeds could not be sterilized, with the methods which we employed, without impairing their vitality. It is a matter of very considerable practical importance to find an agent which, at the same time that it is inexpensive, will effectively sterilize the surface of seeds without harming the germ within. We tried copper sulphate in various concentrations, but found it unreliable and often injurious. In most instances we used a saturated aqueous solution of corrosive sublimate. This is thoroughly efficient wherever it penetrates, but a considerable part of the surface of many, if not most, seeds is covered with a film of air, hard to dislodge, which prevents the sterilizing solution from reaching the spores or bacteria which may be adhering to the seed coats. SCHRÖDER[7] has discussed the

[7] SCHRÖDER, H., Die Wiederstandsfähigkeit des Weizen- und Gerstenkornes gegen Gifte und ihre Bedeutung für die Sterilization. Centralbl. f. Bakt. **28**: 492. 1910.

difficulties in the way of sterilization, and suggests various methods and agents, but for our purposes the solutions of corrosive sublimate were the least objectionable. It is obviously desirable, if sterilization is to be generally practiced, to have a less powerful general poison than corrosive sublimate, at least in concentrated solutions; and experience has shown us that some seeds are so thin-walled or have walls so easily penetrated by the poison that it is only too easy to injure or even to kill them. Several of our experiments gave entirely negative results from this cause. Clover seemed to be particularly susceptible, presumably on account of the small size of the seed and its thin and permeable coat.

Experimental work

We made germination experiments, both in Dewar flasks and in seed beds, on barley, clover, corn (Cory sweet corn), hemp, oats, and wheat. The records of these are in the form of tables, graphs,

TABLE I

BARLEY

Date	Time	Hours elapsed	Room	Max.	Min.	1905	1911
March 8	11:00 A.M.	.....	19°.9 C.	.....	.....	17°.10 C.	17°.00 C.
9	10:00 A.M.	23	17.3	17°.21 C.	16°.22 C.	17.20	17.10
9	5:00 P.M.	30	17.2	.....	.....	17.30	17.35
10	9:00 A.M.	46	17.2	17.16*	16.17	17.50	17.77
10	6:00 P.M.	55	17.2	.....	.....	17.80	18.50
11	9:30 A.M.	70.5	17.1	17.16	16.17	18.30	19.44
11	5:00 P.M.	78	17.1	.....	.....	18.60	20.20
12	9:00 A.M.	94	17.2	17.16	16.06	19.20	22.20
12†	4:00 P.M.	101	17.2	.....	.....	19.50	22.50
12	4:05 P.M.	101	17.2	.....	.....	19.20	22.00
13	9:45 A.M.	118.75	17.1	17.21	16.39	20.20	22.25
13	4:30 P.M.	125	17.0	.....	.....	20.62	22.00
14	9:00 A.M.	142	17.0	.....	.....	21.40	21.40
14	3:00 P.M.	148	17.0	.....	.....	21.40	21.30

*This and other discrepancies between room and maximum temperatures are due to (1) the poor thermometer used to indicate the room temperature, and (2) the fact that the maximum temperatures were calculated from a Weather Bureau pattern thermometer with Fahrenheit scale.

†On the afternoon of March 12, the flasks were opened in order to determine whether any odor was present. None was detected and the contents of the flasks were not disturbed in any way. Nevertheless, the temperature in the flask containing 1911 seed fell steadily, from that time on, as the record shows.

and photographs. Our records will show that, other things being equal, a high temperature within a reasonable length of time is indicative of high germinating power and also of the ability to make

a rapid growth of root and shoot after germination, in other words, of vigor. The following experiments furnished the grounds for this statement.

EXPERIMENT I.—Barley from the University of Wisconsin; 15 grams of the crop of 1905 and a like amount from the crop of 1911, washed with a saturated solution of copper sulphate. Experiment set up March 8, 1912. The data are in table I.

On the eighth day the flasks were opened and emptied. No infection was visible and both lots of seed had germinated freely. It is to be noted, however, that the radicles from the 1911 seed were two or three times as long as those from the 1905 seed. The accompanying graph (fig. 1), constructed from the thermometer readings of table I, indicates the evolution of heat in these two sets of seeds.

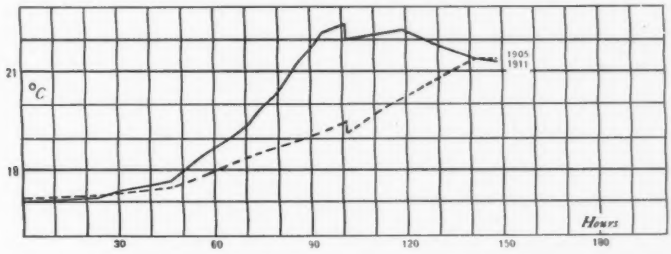


FIG. 1.—Barley (experiment I): 15 grams; broken line, 1905; solid line, 1911

EXPERIMENT II.—Barley from the University of Wisconsin and from Ames, Iowa; 10 grams of each year. On account of the hulls, no sterilization was attempted. Experiment set up as indicated in 1913. The data are in tables II and III.

The fact that the 1909 seeds were very moldy when taken from the flask accounts for the high temperature in that flask. Two other experiments were set up at the same time, but as we were not able to sterilize, the seeds were found to be covered with mold and the unusually high temperatures were not thought worth recording.

It is not of course to be expected that such seeds will show a perfectly regular decrease in heat yield with increasing age. There are too many other factors which may influence their viability. In order correctly to interpret the variations from this regular decrease,

one should know the climatic conditions under which the seed was grown and harvested, and the conditions under which it was afterward stored. Immature seeds are open to the influence of

TABLE II

WISCONSIN BARLEY

Date	Time	1905	1906	1907	1908	1909	1910	1911	Room
April 13	1:00 P.M.	17°.4 C.	17°.5 C.	17°.4 C.	17°.4 C.	17°.6 C.	17°.6 C.	17°.6 C.	17°.0 C.
14	8:00 A.M.	17.6	17.6	17.5	17.6	18.6	18.4	18.2	16.9
14	6:00 P.M.	17.8	17.7	17.5	17.8	19.3	19.2	18.6	16.9
15	8:00 A.M.	17.9	17.8	17.5	18.0	21.1	21.0	19.1	16.9
15	5:15 P.M.	18.0	18.0	17.6	18.2	22.9	23.0	19.6	16.9
16	8:15 A.M.	18.3	18.3	17.6	18.6	23.6	24.0	20.8	16.9
16	5:30 P.M.	18.4	18.5	17.6	18.9	23.3	23.8	20.9	16.9
17	8:00 A.M.	18.7	19.0	17.7	19.4	22.4	23.3	21.0	16.9
18	8:30 A.M.	19.4	19.3	17.9	19.7	21.3	22.7	20.5	16.9
18	6:00 P.M.	19.5	19.4	17.9	19.7	21.0	22.6	20.3	16.9
19	9:00 A.M.	19.5	19.6	18.0	19.6	20.8	22.6	20.1	16.8
19	4:30 P.M.	19.6	19.7	18.05	19.6	20.7	22.7	20.1	16.9
21	8:00 A.M.	19.9	20.5	18.4	19.6	20.6	23.4	20.1	16.9
		2°.5	3°.0	1°.0	2°.2	3°.0	5°.8	2°.5	Increase

The 1910 and 1906 seeds were moldy when taken from the flasks.

TABLE III

BARLEY NO. 202

Date	Time	1909	1910	1911	Room
April 23.....	8:30 A.M.	17°.6 C.	17°.7 C.	17°.6 C.	17°.2 C.
23.....	4:30 P.M.	17.7	17.8	17.7	17.1
24.....	8:00 A.M.	18.0	18.2	18.0	17.2
24.....	4:30 P.M.	18.1	18.4	18.1	17.2
25.....	9:30 A.M.	18.6	19.1	18.6	17.2
25.....	5:00 P.M.	18.9	19.2	18.9	17.3
26.....	12:00 M.	19.7	19.2	19.7	17.3
27.....	10:00 A.M.	20.4	19.2	20.4	17.3
28.....	8:00 A.M.	21.7	19.4	21.7	17.3
28.....	6:00 P.M.	22.5	19.6	22.5	17.3
29.....	9:00 A.M.	24.9	20.1	24.9	17.3
		7°.3	2°.4	2°.1	Increase

environmental conditions to a far greater extent than the fully mature seed (EWART, *loc. cit.*). Even fully matured seeds, when stored under humid conditions, lose their vitality much more quickly than those stored in a dry atmosphere (DUVEL, *loc. cit.*).

The foregoing records have shown that there is a decrease in the amounts of heat liberated by germinating seeds of certain sorts as the seeds increase in age. In order to ascertain whether there is any proportional relationship between the respiratory activity, as indicated by the heat yield, and the germinating power, we still further tested some of the seeds used in the foregoing experiments by setting them out to germinate. Five lots of barley, those from Wisconsin and the no. 202 above, together with three more from Ames, Iowa,

TABLE IV

	Year	Number sprouting April				Per-cent-age spr. 28	Lengths of plumules in cm. April 27–28			
		25	26	27	28		Shortest	Longest	Av.	Percent-age growth
No. 364...	1909	17	25	38	.....	76	1.4	7.8	3.5	43
	1910	16	22	27	.....	54	1.0	8.1	5.5	67
	1911	31	37	46	.....	92	2.4	7.5	4.7	58
No. 294...	1909	19	27	43	.....	86	1.0	7.7	5.3	65
	1910	21	25	32	.....	64	1.0	8.7	6.9	8.5*
	1911	27	38	49	.....	98	1.0	8.9	6.1	75
No. 304...	1909	26	29	41	.....	82	1.3	8.5	6.0	74
	1910	24	24	28	.....	56	0.5	7.9	6.5	80
	1911	38	45	45	.....	90	1.0	9.5	6.6	81
Wisconsin.	1905	17	26	.....	40	80	0.5	8.9	5.3	65
	1906	3	23	.....	29	58	1.2	8.9	5.9	72
	1907	6	12	.....	18	36	1.0	7.6	4.2	51
	1908	26	38	.....	42	84	0.3	10.5	6.8	83†
	1909	41	46	.....	49	98	3.5	10.5	8.1	100
	1910	29	41	.....	47	94	2.0	9.7	6.7	82
	1911	28	30	.....	42	84	2.2	8.4	6.5	80
No. 202...	1909	12	29	.....	40	80	1.6	7.5	5.4	66
	1910	18	35	.....	40	80	3.5	8.1	6.5	80
	1911	18	41	.....	47	94	1.5	9.8	6.5	80

* Length of plumules April 27. † Lengths measured April 28.

were selected for the test. Fifty kernels of each year in each of the five lots were soaked for 24 hours in boiled distilled water. They were then planted in rows in shallow boxes of sand which had previously been steamed for three hours in an Arnold steam sterilizer and allowed to cool. The boxes were then set on the benches in the greenhouse awaiting germination. The date was April 22, 1913. The weather was very warm at this time. This partly accounts for the very quick response of these seeds. The data are in table IV.

The first counting was made three days from the time the seeds were planted; the last, six days from that time. In almost every case the 1911 seeds show the greatest number of seedlings at the

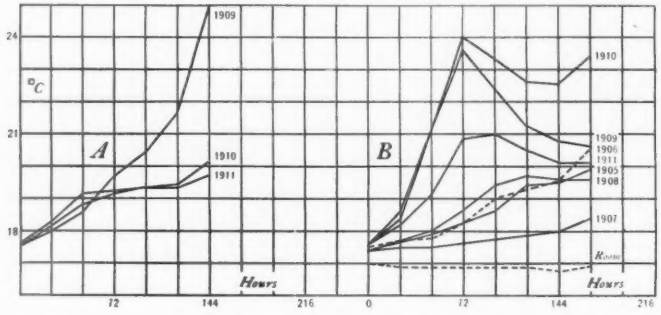


FIG. 2.—Barley in flasks (experiment II): *A*, no. 202; *B*, Wisconsin

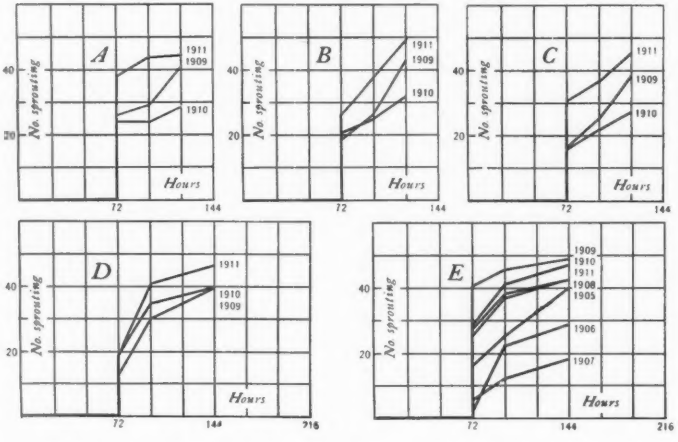


FIG. 3.—Barley in soil: *A*, no. 304; *B*, no. 294; *C*, no. 346; *D*, no. 202; *E*, Wisconsin.

first as well as at the last counting, and here also the 1910 seeds show lowered vitality, especially when compared with 1909. In the percentage of growth of the seedlings, the same decrease with age is evident.

For the sake of comparison, the accompanying curves are given (figs. 2, 3), but attention should be called again to the fact that, owing to the hulls, no attempt at sterilization was considered worth while, and hence there was very considerable molding in the Dewar flasks, with a corresponding rise in temperature independent of the heat yielded by the germinating barley. That this is the case will be more plainly shown in subsequent experiments and figures.

In spite of the various defects in this experiment, it is clear not only that the germinating power of seeds declines from year to year, but that this is indicated and can be tested by ascertaining the heat yield in such a good insulator as silvered Dewar flasks. Furthermore, inspection of the tables will show not only that the youngest seeds show the highest percentage of germination and the greatest heat yield, but they respond most quickly to the influence of conditions favorable to germination. They reach the maximum percentage of germination and maximum temperature sooner than older seed.

EXPERIMENT III.—Red clover (*Trifolium pratense*); 25 grams of seed from the crop of 1904 and the same amount from 1911; sterilized by washing with a concentrated aqueous solution of corrosive sublimate which was removed by repeated rinsings with boiled distilled water; placed in two sterile flasks and covered with boiled distilled water previously reduced to room temperature. At the end of 18 hours the water was poured off and the plugs carefully replaced in the necks of the flasks. The experiment was set up on February 20, 1912. The temperature record is given in table V.

TABLE V

Date	Time	Hours elapsed	Room	Max.	Min.	1904	1911
February 20	4:00 P.M.	.....	17°6 C.	.....	.....	18°20 C.	18°10 C.
21	9:30 A.M.	17.5	17.8	17°16 C.	16°39 C.	18.10	18.20
21	4:30 P.M.	24.5	.....	.....	.....	17.90	18.60
22	10:00 A.M.	42.0	17.6	17.05	16.50	17.80	20.20
22	5:30 P.M.	49.5	.....	.....	.....	17.70	21.70
23	9:30 A.M.	66.5	17.7	17.16	16.39	17.70	24.50
23	5:30 P.M.	74.5	18.0	.....	.....	17.70	25.40
24	9:30 A.M.	91.5	17.6	17.11	16.50	18.00	26.30
24	5:30 P.M.	99.5	17.7	.....	.....	18.40	26.45
25	9:30 A.M.	116.0	17.7	17.16	16.50	24.70	27.40
25	3:00 P.M.	122.0	17.7	.....	.....	25.80	27.50

As table V shows, and as graphically displayed in fig. 4, there was a very marked difference in the heat liberated by the two sets of seeds up to the fifth day. At this time the temperature in the 1904 flask rose very rapidly. Upon opening the flasks, the cause of this sudden rise was at once seen to be the active fermentation, the odor of which was evident, and the contents of the flask were found to be covered with *Penicillium*. The accuracy with which the heat yield indicates the germinating power of the seeds is shown by fig. 5, in which the contents of the two flasks are photographed in the Petri dishes into which they were emptied for this purpose. Not

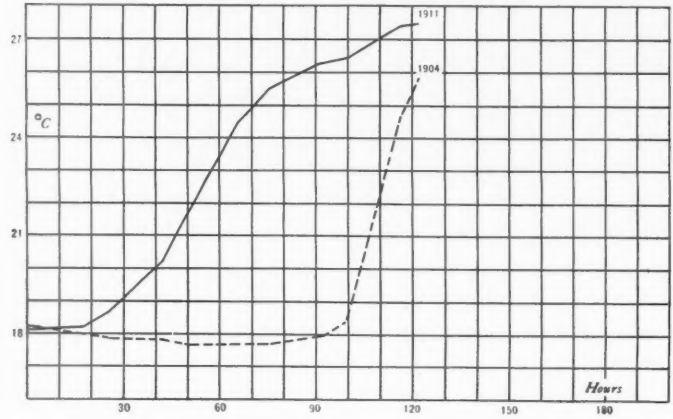
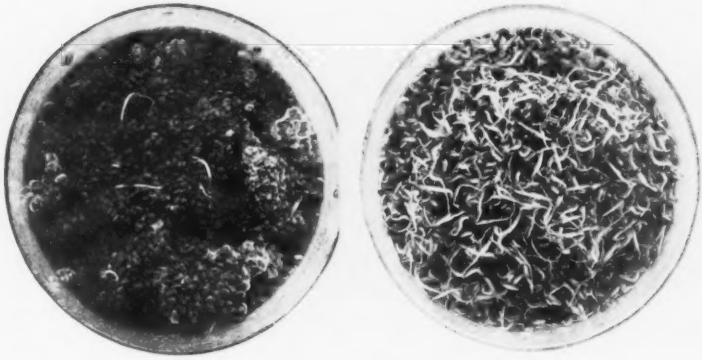


FIG. 4.—Red clover (experiment III): 25 grams; broken line, 1904; solid line, 1911.

more than 1 per cent of the whole quantity of 1904 seed showed any sign of germination. On the other hand, practically all of the 1911 seed had germinated and the seedlings were growing vigorously. In this flask merely a very slight trace of mold was present. I do not see that the inference that the faulty germination of the 1904 seed was due to the amount of mold and bacteria upon it is at all justified by the evidence, but rather that the sterilization had resulted in killing all but a few spores of the mold, and that some time had to elapse before these could produce any considerable amount of mycelium and liberate any considerable amount of heat.

EXPERIMENT IV.—Cory sweet corn, white cob, bought of C. C. Morse & Co., seedsmen, San Francisco, in 1908 or 1909 and 1911 or 1912, and purporting to be of the crops of 1908 and 1911, respectively; 30 grams of each, thoroughly washed in a 10 per cent aqueous solution of copper sulphate and then rinsed in boiled distilled



A *B*

FIG. 5.—Red clover: *A*, 1904; *B*, 1911

water at room temperature, were put in silvered Dewar flasks and soaked for 24 hours in boiled distilled water. This was poured off when the first temperature reading was taken. The experiment was set up on March 15, 1912. The data are given in table VI.

TABLE VI

Date	Time	Hours elapsed	Room	Max.	Min.	1908	1911
March 15	12:30 P.M.	.....	17°6 C.	.....	.....	17°00 C.	17°10 C.
16	11:30 A.M.	23	16.9	17°21 C.	16°01 C.	17.00	17.15
17	10:30 A.M.	46	17.0	17.16	16.01	16.90	18.70
18	9:30 A.M.	69	17.0	17.16	16.06	16.90	20.75
18	5:00 P.M.	76.5	17.0	.....	.....	17.00	21.45
19	9:00 A.M.	92.5	17.0	17.16	16.06	17.75	23.00
19	5:00 P.M.	100.5	17.0	.....	.....	18.25	23.70
20	9:30 A.M.	117	17.2	17.16	16.06	18.75	24.45
20	4:30 P.M.	124	17.0	.....	.....	18.56	24.45
21	9:00 A.M.	140	17.0	17.16	16.01	18.40	24.30

On opening the flasks, both sets of seeds were found to be infected with mold, but with more on the older seeds than on the

younger ones; 24 per cent of the 1908 corn had germinated at the end of the experiment; the radicles were short as well as few. Practically 100 per cent of the 1911 corn germinated and the radicles were long and vigorous. In fig. 6 are given the curves made from the temperature data of table VI. This experiment, in addition to being very clear and striking in its indication of the deterioration which takes place on keeping seed even for the short space of three years, is of special interest because it furnishes a test of the value of commercial seed.

EXPERIMENT V.—Hemp seed, from Ames, Iowa, of the crops of 1907 and 1911, and of 1908 and 1910; 5.5 grams of each lot were

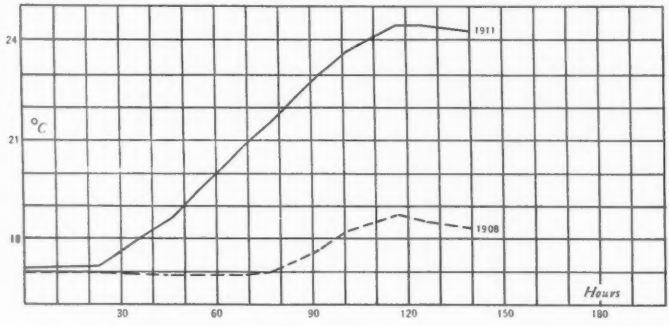


FIG. 6.—Cory corn (sweet) (experiment IV): 30 grams; broken line, 1908; solid line, 1911.

washed with concentrated aqueous solution of corrosive sublimate, thoroughly rinsed with boiled distilled water, and soaked for the usual length of time. The experiment with 1907 and 1911 seed was set up on May 1, that on the 1908 and 1910 seed on May 13, 1912. The data are given in table VII.

It is obvious that, because of the small amount of seed used, namely, 5.5 grams, the rise in temperature would be less than when larger quantities were used; but it is interesting and significant that only a small sample need be used to test the quality of seed, provided only that the insulator be a good one. Thus we have a rise of 5°.35 C. taking place in the flask containing 1911 seed, whereas the temperature in the flask containing 1907 seed merely fluctuated

with the temperature of the room. On opening the flasks a corresponding difference was revealed in the condition of the two sets of seeds. The 1911 lot had germinated very freely, and showed rather remarkable growth. Many radicles were 3–4 cm. long, and the cotyledons had emerged in nearly all the seedlings. On the other hand, only three of the 1907 seeds had germinated, less than 1 per cent. There was no mold in the 1911 seed, whereas the older seed

TABLE VII

Date	Time	Hours elapsed	Room	1907	1911	1908	1910
May 1...	4:00 P.M.	.....	18°0 C.	19°80 C.	20°55 C.	.....	.....
2...	2:30 P.M.	21.5	17.5	17.60	18.35	.....	.....
3...	9:30 A.M.	40.5	17.5	16.70	17.35	.....	.....
3...	5:30 P.M.	48.5	17.5	16.75	17.40	.....	.....
4...	8:00 A.M.	63.0	17.6	16.80	17.75	.....	.....
4...	5.30 P.M.	72.5	17.6	16.80	18.10	.....	.....
5...	9:30 A.M.	88.5	17.6	16.85	18.90	.....	.....
5...	4:30 P.M.	95.5	17.7	16.90	19.40	.....	.....
6...	9:30 A.M.	112.5	17.8	17.15	20.60	.....	.....
6...	3:30 P.M.	118.5	18.2	17.30	20.90	.....	.....
7...	8:30 A.M.	135.5	17.8	17.20	21.50	.....	.....
7...	4:30 P.M.	143.5	18.0	17.20	21.70	.....	.....
9...	10:00 A.M.	181.0	18.0	17.20	22.70	.....	.....
10...	9:30 A.M.	204.5	18.0	16.75	21.90	.....	.....
11...	9:30 A.M.	228.5	18.0	17.30	21.40	.....	.....
13...	12:00 M.	.....	18.0	.....	.....	27°80 C.	28°00 C.*
14...	9:30 A.M.	21.5	18.0	.....	.....	21.70	20.80
14...	3:30 P.M.	27.5	18.0	.....	.....	18.60	17.90
15...	8:30 A.M.	44.5	18.0	.....	.....	17.90	17.50
16...	8:00 A.M.	68.0	18.0	.....	.....	17.90	17.80
16...	4:00 P.M.	76.0	18.2	.....	.....	18.00	18.00
17...	9:30 A.M.	93.5	18.0	.....	.....	18.10	19.10
17...	3:30 P.M.	99.5	18.0	.....	.....	18.10	19.80
18...	9:30 A.M.	117.5	18.0	.....	.....	18.40	22.00
20...	5:30 P.M.	173.0	18.0	.....	.....	19.80	21.10
22...	9:30 A.M.	214.0	18.0	.....	.....	20.20	20.00

*In setting up this experiment, it was necessary to prepare a fresh supply of boiled distilled water. This did not have time to cool to room temperature, and hence the high initial temperatures and the immediate drop to room temperature.

had molded considerably. As table VII shows, the records of the 1910 and 1908 seeds are intermediate between those of the 1911 and 1907 seeds. About 95 per cent of the 1910 seeds germinated, but of the 1908 seed not over 40 per cent germinated. The seedlings of both of these latter sets were vigorous, as is shown by fig. 7. We believe that the high initial temperature to which the seeds of these two latter sets were exposed stimulated respiration in both lots, and

that the temperature record is somewhat higher than it would have been under perfectly normal and proper conditions.

From this experiment it is evident that a small sample of seed in a Dewar flask of good quality may be used with confidence to indicate the germinating power of a much larger quantity. At the same time that we are fortunate in knowing the ages of the seeds used in this experiment, we cannot know that the seeds were equally mature when they were harvested in the several seasons, and we can only believe that they were equally well kept. But be these things as they may, this experiment demonstrates the feasibility of ascertaining the germinating power of a given lot of seed, whatever

A *B*

FIG. 7.—Hemp: *A*, 1908; *B*, 1910

its age and experience may have been, and, furthermore, shows that this is possible in a short time and even with small quantities of material. But on the assumption that these four lots of seed differed from one another only in age, an assumption which we cannot make with any assurance, it is noticeable that the germinating power falls abruptly; thus the germinating power of the 1911 seed in 1912 was approximately 100 per cent, of the 1910 seed approximately 95 per cent, of the 1908 seed 40 per cent, and of the 1907 seed less than 1 per cent. We did not have any 1909 seed, and we regret this. The results recorded in table VII are graphically represented in fig. 8.

EXPERIMENT VI.—Oats from the University of Wisconsin, from the Experimental Farm at Davis, California, under the direction of the University of California, and from Ames, Iowa, were used in different quantities, as recorded below. In our early tests we attempted to sterilize the material, but experience showed this to be futile, at least under the ordinary conditions. It is to be noted, however, that in almost every instance, the amount of fungous infection increased with the age of the seeds. This is indicated in all of our experiments. In our first test, 20 grams of oats of the crops of 1904 and of 1911 from the University of Wisconsin were

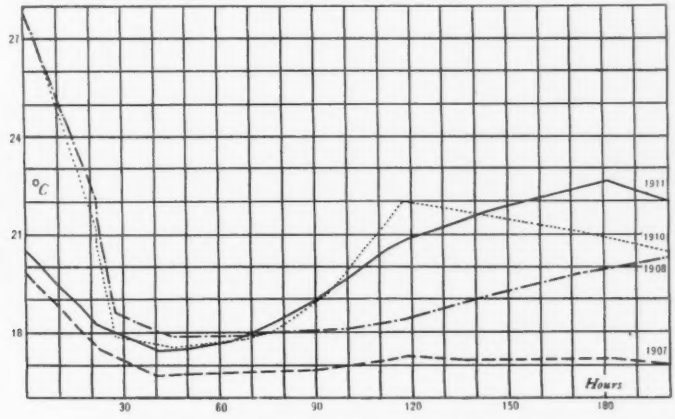


FIG. 8.—Hemp (experiment V): 5.5 grams; broken line, 1907; broken and dotted line, 1908; dotted line, 1910; solid line, 1911.

washed with a concentrated aqueous solution of corrosive sublimate, rinsed with boiled distilled water, and then soaked as before in the flasks and the water presently drawn off. The data are in table VIII.

On opening the flasks recorded in table VIII, one saw that 24.4 per cent of the 1904 seed had germinated, and of these seedlings not more than 2 or 3 per cent had radicles more than 3–4 mm. in length. A trace of blue mold was present. On the other hand, practically all of the 1911 seed had germinated, the radicles were long and vigorous, and there was no evidence of infection.

For purposes of contrast, and as indicating the direct connection between the quality of the seed and the amount of heat which it will liberate on germination, the following record is given in a separate table (IX), instead of being combined, as in some of the other cases, with others. Oats of the seasons of 1907 and 1911, from Davis

TABLE VIII

Date	Time	Hours elapsed	Room	1904	1911
February 27.....	4:00 P.M.	.....	17°.8 C.	18°.30 C.	18°.20 C.
28.....	4:00 P.M.	24.0	17.7	17.90	17.90
29.....	9:00 A.M.	41.0	17.6	17.85	17.80
29.....	3:30 P.M.	47.5	17.6	17.85	17.80
March 1.....	10:00 A.M.	66.0	17.6	18.00	18.15
1.....	6:30 P.M.	74.5	17.8	18.05	18.40
2.....	11:00 A.M.	91.0	17.7	18.20	19.00
2.....	6:00 P.M.	98.0	17.6	18.25	19.35
3.....	9:30 A.M.	113.5	17.5	18.40	20.10
4.....	9:30 A.M.	137.5	17.4	18.70	21.40
4.....	5:30 P.M.	145.5	17.6	18.70	22.00
5.....	9:00 A.M.	162.0	17.4	19.10	23.10
5.....	4:00 P.M.	169.0	.....	19.30	23.50
6.....	3:00 P.M.	192.0	17.6	20.10	24.38

California, were used. Much dirt and chaff, and many broken grains were present in the lot, but these were removed as carefully as possible. The cleaned residue was then treated with corrosive sublimate solution, and washed with boiled distilled water as before. Without these extra precautions, the results would have been

TABLE IX

Date	Time	Hours elapsed	Room	1907	1911
April 3.....	3:30 P.M.	.....	18°.2 C.	18°.30 C.	17°.50 C.
4.....	4:30 P.M.	25	17.5	17.70	17.60
6.....	11:30 A.M.	68	17.6	18.00	18.50
8.....	9:30 A.M.	114	.....	19.90	21.65
9.....	10:30 A.M.	139	17.6	20.70	24.75
9.....	4:30 P.M.	145	17.5	20.60	25.45
10.....	2:30 P.M.	167	17.5	20.90	27.50

entirely unreliable. The thoroughness with which one of us cleaned the material is indicated by the figures in table IX, the record of 30 grams of seed from each lot.

In figs. 9 and 10 are shown the curves constructed on the basis of the records of tables VIII and IX. Allowing for the difference in

the weights of the two samples and a correspondingly steeper curve. the difference in these two curves is indicative of the difference in the cleanliness, and the corresponding goodness of the two sets or lots of seeds. It is to be noted, also, that both sets of seeds in this

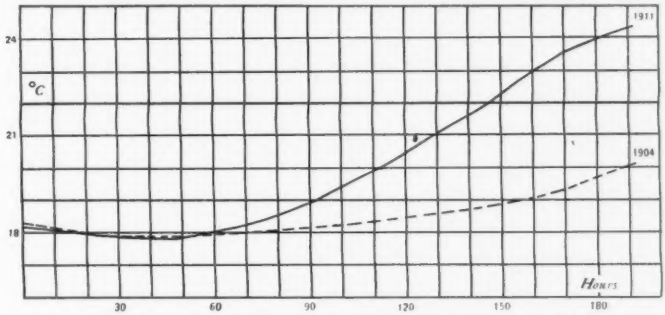


FIG. 9.—Oats (experiment VI): 20 grams; broken line, 1904; solid line, 1911

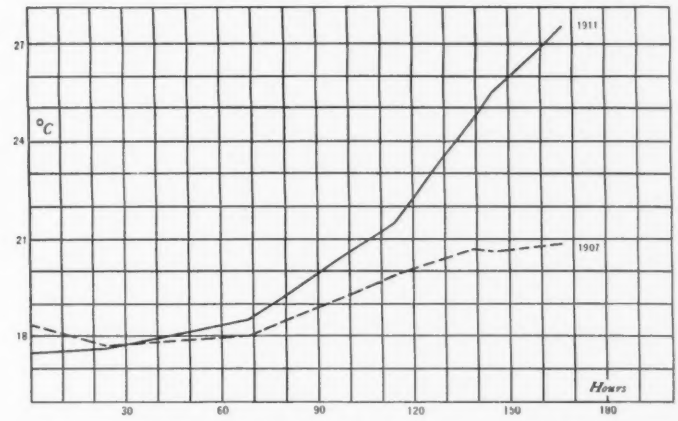


FIG. 10.—Oats (no. 339): 30 grams; broken line, 1907; solid line, 1911

latter test were badly infected with mold. Of the 1907 seeds less than half had germinated; but the 1911 seeds showed nearly 100 per cent germination.

With these records, which show the behavior of certain varieties of oats during the winter following the summer in which they grew,

we should compare others made a year later. Except where explicitly stated otherwise, the quantities used were 10 grams of seed. These were selected as well as possible to insure soundness, hulled, washed quickly in a saturated aqueous solution of corrosive

TABLE X

OATS NO. 444

Date	Time	Room	1909	1910	1911
March 20....	9:00 A.M.	16°.5 C.	16°.9 C.	17°.0 C.	17°.0 C.
20....	4:30 P.M.	16.4	17.8	17.6	18.0
21....	9:00 A.M.	16.4	18.7	18.3	19.4
21....	4:30 P.M.	16.4	19.3	18.6	20.5
22....	10:30 A.M.	16.4	21.9	19.9	25.0
22....	4:00 P.M.	16.3	23.0	20.4	26.1
23....	11:00 A.M.	16.3	23.3	22.5	24.7
24....	11:00 A.M.	16.2	20.3	23.3	20.7
Increase in temperature between first and highest readings.....			6°.4	6°.3	9°.1

TABLE XI

WISCONSIN OATS

Date	Time	Room	1904	1905	1906	1907	1908	1909	1910	1911
March 26..	6:00 P.M.	16°.2C.	16°.2C.	16°.6C.	16°.5C.	16°.5C.	16°.8C.	16°.7C.	16°.7C.	16°.7C.
27..	9:00 A.M.	16.1	16.9	17.1	16.8	16.9	17.8	17.6	17.2	17.5
27..	4:00 P.M.	16.1	17.0	17.2	16.8	17.0	18.2	18.0	17.4	17.85
28..	9:00 A.M.	16.1	17.3	17.8	17.1	17.4	20.2	19.4	18.0	19.3
28..	4:30 P.M.	16.1	17.6	18.2	17.2	17.7	21.9	20.6	18.4	20.4
29..	9:00 A.M.	16.2	18.3	19.6	17.7	18.5	26.8	14.9	19.6	24.0
29..	6:30 P.M.	16.3	18.9	20.5	18.0	19.2	26.8	28.4	20.5	25.0
30..	11:00 A.M.	16.3	19.8	20.5	18.7	19.7	24.3	30.0	20.8	23.9
30..	7:30 P.M.	16.4	19.9	20.4	18.9	19.7	23.1	28.6	20.6	23.1
31..	9:00 A.M.	16.4	19.7	20.0	18.9	19.6	21.7	26.3	20.3	22.1
Increase in temperature between first and highest readings.....			3°.3	3°.9	2°.4	3°.2	10°.0	13°.3	4°.2	8°.3

sublimate, and rinsed four times in sterile distilled water. They were then put into the Dewar flasks, which had been previously sterilized, together with the thermometers, and covered for 24 hours with sterile distilled water. At the end of this time the water was poured off. The data are given in tables X–XIV.

Inspection of table XI will show that the seeds of the crops of 1909 and of 1911 gave considerably higher temperatures than the older seeds of the four years preceding, but the results are not so regular as an average of similar experiments on similar seeds of this and other sorts might give. Not knowing the maturity of the seed when harvested, the manner of its curing and storage, and various other factors which might affect its vigor, we are dependent, in all of these cases, upon the facts of age and vigor as shown by Dewar flask and seed bed. We cannot further account for the differences.

TABLE XII

OATS NO. 293

Date	Time	Room	1907	1908	1909	1910	1911
April 2.....	3:00 P.M.	16°.7 C.	17°.2 C.	17°.3 C.	17°.3 C.	17°.2 C.	17°.3 C.
3.....	9:00 A.M.	16.7	17.6	17.7	17.6	17.8	18.4
3.....	3:00 P.M.	16.6	17.7	17.85	18.75	18.1	18.9
4.....	9:00 A.M.	16.7	18.4	18.75	18.5	19.2	21.8
4.....	5:00 P.M.	16.7	18.8	19.35	19.0	20.0	24.0
5.....	9:30 A.M.	16.6	20.2	20.5	19.4	21.2	27.8
5.....	5:00 P.M.	16.7	20.2	20.6	19.4	21.4	27.4
6.....	8:00 A.M.	16.7	20.1	20.3	19.15	20.9	24.4
6.....	5:00 P.M.	16.7	19.8	19.9	18.9	20.5	22.9
7.....	8:00 A.M.	16.6	19.2	19.3	18.6	19.8	21.6
7.....	5:00 P.M.	16.7	19.1	19.15	18.5	19.6	21.2
8.....	8:00 A.M.	16.9	18.7	18.6	18.3	18.8	20.1
Increase in temperature between first and highest readings				4°.7	5°.9	4°.2	3°.1

In this and some of the other tests, the first temperature reading in the Dewar flasks was 0°.5–0°.6 higher than the room temperature. This points to the fact that in the preliminary stages of germination, while the seeds were soaking in water, heat was also being liberated. The rate of heat liberation at different stages in germination is not uniform. This one of us will show elsewhere, for it has little to do with the special topic of this paper.

Table XIII shows irregularities which we are unable to account for, since we are unacquainted with the experience of the seeds before they came into our hands; but we include the table for the sake of completeness.

The record of the behavior of seeds of the same ages and varieties in a seed bed, that is, under the usual conditions for germination,

will show how well the viability of the seeds is indicated by their heat yields within a comparatively short time after germination is

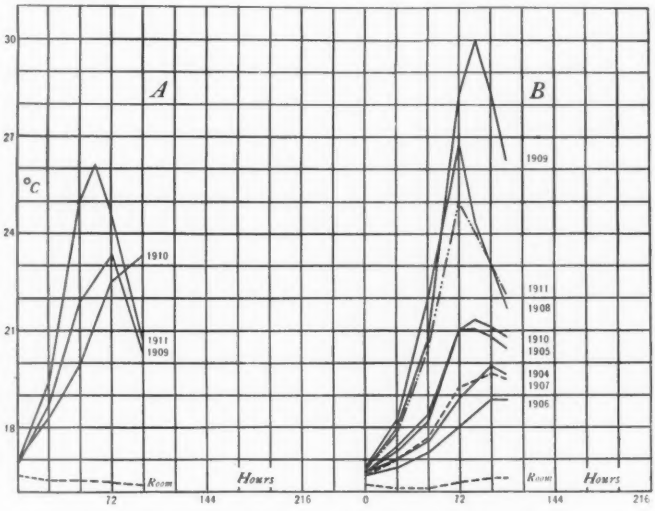


FIG. 11.—Oats in flasks: *A*, no. 444; *B*, Wisconsin

started in Dewar flasks. Fifty kernels were selected from each lot, but on account of the hulls, no attempt was made to sterilize them.

TABLE XIII

OATS NO. 339

Date	Time	Room	1907	1909	1910	1911
April 3....	3:00 P.M.	16°6 C.	17°2 C.	17°2 C.	17°3 C.	17°2 C.
4....	9:00 A.M.	16.7	17.9	18.2	18.1	17.7
4....	5:00 P.M.	16.7	18.1	18.6	18.4	18.0
5....	9:30 A.M.	16.6	18.7	20.2	19.2	18.6
5....	5:00 P.M.	16.7	19.0	21.2	19.6	19.0
6....	8:00 A.M.	16.7	20.2	23.0	20.8	19.8
6....	5:00 P.M.	16.7	21.1	23.0	21.3	20.2
7....	8:00 A.M.	16.6	22.6	21.8	21.5	20.3
7....	5:00 P.M.	16.7	22.9	21.2	21.4	20.2
8....	8:00 A.M.	16.9	20.2	19.3	19.5	18.8
Increase in temperature between first and highest readings.....			4°7	5°9	4°2	3°1

TABLE XIV

Oats	Year	Number sprouting February				Percentage sprouting	Lengths of plumules Feb. 8–9 in cm.			Percentage of growth
		3	4	6	8		Shortest	Longest	Average	
White Probster...	1872	0	0	0	0	0	.....	.....	.....	.....
	1876	0	0	0	0	0	.....	.....	.....	.....
Wisconsin....	1877	0	0	0	0	0	.....	.....	.....	.....
	1904	24	38	44	48	96	2.2	12.1	9.12	75
	1905	31	39	42	42	84	1.0	11.8	8.10	67
	1906	5	27	41	46	92	3.1	11.1	7.96	66
	1907	19	27	35	39	78	1.6	11.3	8.44	70
	1908	37	49	49	49	98	8.2	11.9	10.50	87
	1909	30	43	47	48	96	3.7	12.4	9.22	76
	1910	8	25	44	44	88	3.0	12.2	8.03	66
	1911	34	46	50	50	100	7.4	13.3	11.33	94
No. 451.	1907	27	33	37	38	76	2.8	12.9	9.98	82
	1909	39	46	48	49	98	2.6	13.5	10.87	90
	1910	33	37	37	37	74	6.2	15.9	12.04	100
	1911	43	49	49	49	98	8.4	15.9	11.56	96
No. 286.	1907	25	27	28	28	56	7.6	14.6	11.29	93
	1909	34	44	45	46	92	3.9	13.5	11.08	92
	1910	30	40	40	47	82	4.7	14.5	9.60	79
	1911	38	46	47	47	94	5.8	16.0	10.55	39
No. 444.	1909	19	33	45	47	94	3.7	12.6	10.34	85
	1910	18	30	35	39	78	1.7	14.5	9.39	77
	1907	27	40	42	44	88	3.8	13.6	9.05	75
No. 293.	1911	18	37	44	50	100	2.9	14.2	10.13	84
	1908	20	33	42	43	86	5.2	16.2	11.51	95
	1909	14	36	48	49	98	6.4	15.0	10.88	90
	1910	21	40	47	47	94	5.4	16.8	10.92	90
	1911	19	40	50	50	100	6.7	16.3	11.42	94
No. 339.	1907	16	28	35	36	72	3.7	13.4	10.20	84
	1909	5	27	37	41	82	2.3	11.9	9.38	77
	1910	2	11	27	33	66	2.1	12.6	8.37	69
	1911	9	30	49	50	100	3.5	6.2	9.99	82

These were planted in boxes of sterilized sand in the greenhouse, on January 28 and 29, 1913.

Comparison of the behavior of oats of different ages as recorded in tables X–XIV is rendered somewhat easier by figs. 11–14. In

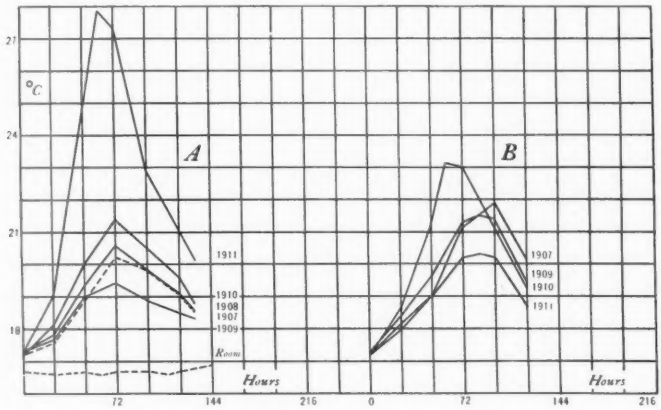


FIG. 12.—Oats in flasks: *A*, no. 293; *B*, no. 339

these it is plainly shown that the vitality and viability of these seeds is indicated more promptly by the temperatures yielded in Dewar flasks than in seed beds, and that what may properly be called the "normal" temperature for the species is developed, other things

TABLE XV

Date	Time	Hours elapsed	Room	1907 (no. 1746)	1911
April 3.....	3:30 P.M.	.....	18°.2 C.	16°.80 C.	17°.10 C.
4.....	4:30 P.M.	25	17.5	16.90	17.50
6.....	11:30 A.M.	68	17.6	18.60	20.50
8.....	9:30 A.M.	114	.....	23.80	28.15
9.....	10:30 A.M.	139	17.6	26.50	28.00
9.....	4:30 P.M.	145	17.5	26.35	27.70
10.....	2:30 P.M.	167	17.5	25.15	27.15

being equal, by the youngest and freshest seed. This will come out still more plainly in the following experiment with wheat.

EXPERIMENT VII.—Wheat, from the University of California, the University of Wisconsin, and the College of Agriculture at Ames

Iowa, was used in the various quantities recorded below and treated in the various ways there indicated. Our first test was made in April 1912, with 30 grams of seed from the Experimental

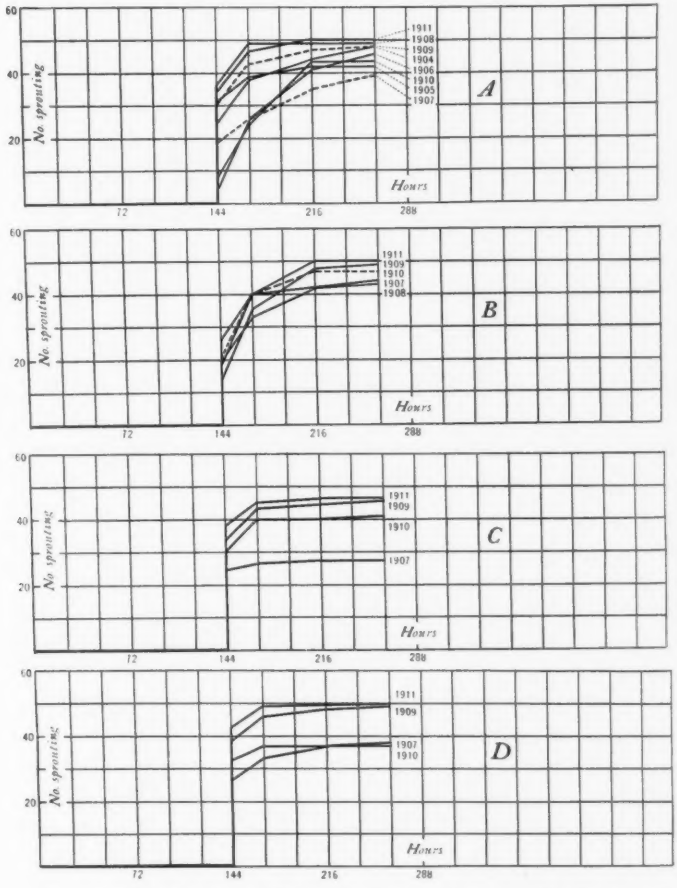


FIG. 13.—Oats in soil: *A*, Wisconsin; *B*, no. 293; *C*, no. 286; *D*, no. 451

Farm of the University of California, at Davis, California. The seed was not as clean and whole as one could wish, and there was too much chaff with it. The material was treated with a concen-

trated aqueous solution of corrosive sublimate and then duly washed in sterile distilled water. The record is given in table XV. Upon opening the flasks, the wheat was found to be quite sweet and free from mold. The 1911 seed showed practically 100 per cent germination. The 1907 seed, on a rough estimate, showed only 60 per cent germination. Both lots of seedlings were growing vigorously.

The next test was made upon four sets of wheat seeds, from the same source as the preceding and of the same ages; 30 grams of seed were used in each lot, and all were carefully washed with an

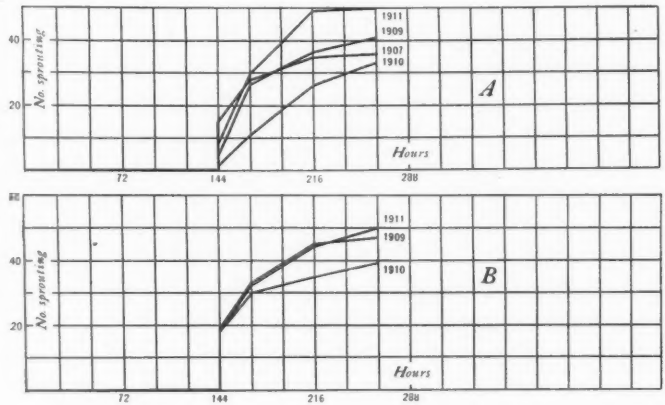


FIG. 14.—Oats in soil: *A*, no. 339; *B*, no. 444

aqueous solution of copper sulphate, saturated at room temperature, and rinsed as usual with sterile water. As the results show, the copper sulphate solution was quite ineffective as an antiseptic. The record is given in table XVI.

As these last temperatures indicate, the seed was too badly infected with mold (*Mucor*) to give the experiment any other significance than this, namely, that germinating seeds, as well as the higher animals, have temperatures which may be considered "normal" or characteristic; and if the seed under test does not show this temperature, or one not far removed from it, one is justified in concluding that there is something wrong with it. If the

temperature is abnormally high, this is due to other organisms also liberating heat in the insulator; if abnormally low, the seed itself is weak. In many cases, at least, this weakness is due to age.

Through the courtesy of Professor PAMMEL, of Ames, Iowa, we had a number of seeds of quite considerable age. We made preliminary germination tests of these while our Dewar flasks were in use, and found that none of the seeds which we had from the crops of 1871 to 1887, inclusive, would germinate under the conditions of our germination boxes. We cannot say that no preservative had been applied to them, but their behavior is entirely consistent with that reported by BECQUEREL and by EWART. BECQUEREL (*loc. cit.*) found that wheat only 34 years old could no longer germinate. EWART (*loc. cit.*) found that out of 750 seeds of wheat 16 years old

TABLE XVI

Date	Time	Hours elapsed	Room	1907 (no. 114)	1911	1907 (no. 639)	1911
April 16.....	3:00 P.M.	.....	18°0 C.	.....	.....	.....	.....
17.....	3:00 P.M.	24	18.0	19°10 C.	19°45 C.	20°00 C.	18°60 C.
18.....	2:30 P.M.	47.5	18.0	18.00	19.20	19.90	19.15
19.....	12:00 M.	69.5	18.0	18.40	21.00	21.35	21.20
20.....	10:30 A.M.	92	17.5	19.00	24.25	23.40	24.60
21.....	10:30 A.M.	116	17.5	21.40	31.00	27.00	26.95
23.....	2:00 P.M.	167.5	.....	38.80	44.20	37.00	42.90

only 8 per cent could sprout, while in other experiments wheat 12–13 years old showed no vitality. We tested, therefore, only two lots of these oldest seeds in Dewar flasks and, owing to the age and corresponding value of the material, used only small quantities; 5 grams of each of these two lots were carefully selected, cleaned, washed with a sterilizing solution, and then rinsed thoroughly with sterile water. The record is given in table XVII.

From these figures it is apparent that one can ascertain whether seeds will germinate under ordinary conditions by moistening them duly and keeping them for a time under known conditions of temperature in Dewar flasks. If, within a reasonable length of time there is no rise in temperature or a rise which does not indicate a "normal" temperature for the species concerned, one may conclude that the seed will not germinate. It goes almost without

saying that the time which must elapse before such a conclusion is justified varies with the species, as does the "normal" temperature.

TABLE XVII

Date	Time	Room	Talaverra spring wheat, 1871	California white spring wheat, 1872
December 12....	11:30 A.M.	19°.0 C.	18°.1 C.	17°.6 C.
13....	1:30 P.M.	19.0	18.1	17.6
14....	9:00 A.M.	18.75	18.1	17.6
14....	4:30 P.M.	18.75	18.1	17.6
15....	4:00 P.M.	18.5	18.1	17.6
16....	1:00 P.M.	18.5	18.0	17.6
17....	2:00 P.M.	18.5	18.0	17.5
18....	12:00 M.	18.5	17.95	17.5
20....	3:00 P.M.	19.0	17.9	17.4

Table XVIII records the temperatures developed in silvered Dewar flasks by three lots of wheat of the same variety, marked no. 791, of the crops of 1909, 1910, 1911; 10 grams of each lot were

TABLE XVIII

Date	Time	Room	1909	1910	1911
February 5..	1:30 P.M.	17°.5 C.	16°.7 C.	16°.7 C.	16°.8 C.
6..	10:00 A.M.	17.0	16.7	16.8	17.3
6..	1:30 P.M.	17.0	16.8	16.9	17.4
6..	4:30 P.M.	17.0	16.9	17.0	17.4
7..	8:30 A.M.	17.0	17.0	17.2	17.7
7..	5:30 P.M.	17.25	17.1	17.3	18.3
8..	9:30 A.M.	17.0	17.3	17.6	19.2
8..	1:30 P.M.	17.25	17.3	17.7	19.4
8..	4:30 P.M.	17.0	17.4	17.7	19.5
9..	2:00 P.M.	17.25	17.7	18.1	20.0
10..	8:30 A.M.	17.0	18.1	18.6	19.7
10..	4:00 P.M.	17.0	18.2	18.7	19.6
11..	8:30 A.M.	17.0	18.3	18.9	19.3
11..	4:30 P.M.	17.0	18.3	19.0	19.2
12..	8:30 A.M.	17.0	18.3	19.0	18.9
12..	4:30 P.M.	17.0	18.2	19.0	18.8
13..	8:30 A.M.	17.0	18.1	18.8	18.7
Increase in temperature between first and highest readings.....			1°.6	2°.3	3°.2

used, being washed and soaked in the usual ways. As the table shows, the rise in temperature of the 1911 seed was much more prompt and rapid than in the other lots, and more rapid in the seed of 1910 than in that of 1909. The maximum temperatures

increased correspondingly regularly from 1909 to 1911, and the maximum is reached more than 42 hours earlier in the 1911 seed than in the other two. In this connection it should be stated that, as will be shown later, the comparative viability of the different lots of seed is indicated in the Dewar flasks within 24–48 hours, whereas the seed planted in soil showed nothing within 72 hours. Had there been older seed than that of 1909, the delay in soil would have been still greater in proportion. The data of the flask test are given in table XVIII.

The increasing heat yield, indicated in the last line of table XVIII, is quite as striking in regularity as the increasing percentage of germination for the crops of the years 1909, 1910, 1911, as recorded in the table of germinations following. This is shown again, and for another variety of wheat, in table XIX.

TABLE XIX

Date	Time	Room	1909	1910	1911
February 6..	1:30 P.M.	17°0 C.	16°6 C.	16°3 C.	16°7 C.
6..	4:30 P.M.	17	16.8	16.4	16.9
7..	8:30 A.M.	17	17.1	16.5	17.3
7..	5:30 P.M.	17.25	17.2	16.65	17.5
8..	9:30 A.M.	17	17.5	16.8	17.9
8..	1:30 P.M.	17.25	17.5	16.8	18.0
8..	4:30 P.M.	17	17.6	16.9	18.0
9..	2:00 P.M.	17.25	17.8	17.0	18.5
10..	8:30 A.M.	17	18.2	17.2	19.1
10..	4:00 P.M.	17	18.3	17.3	19.3
11..	8:30 A.M.	17	18.5	17.4	19.8
11..	4:30 P.M.	17	18.6	17.5	19.9
12..	8:30 A.M.	17	18.7	17.6	19.9
12..	4:30 P.M.	17	18.8	17.7	19.9
13..	8:30 A.M.	17	18.8	17.75	19.8
Increase in temperature between first and highest readings.....			2°2	1°45	3°2

Ten grams of each harvest, 1909 to 1911, inclusive, of wheat no. 98, were treated in the usual way and placed in Dewar flasks. The figures are given in table XIX.

For some unknown reason, the seeds of the 1910 lot started at a disadvantage, the temperature in the flask in which they were contained (flask no. 5) being 0°3–0°4 lower than in the other two. Nevertheless, the temperatures behave as in the preceding experi-

ments, rising more rapidly and to a higher point in the flask containing the freshest seed, and the rise beginning more promptly among the freshest seed, the other seeds lagging more and more according to their ages. In 24 and 48 hours marked differences in viablility are indicated in the flasks which did not appear in the seed beds until 72 hours had elapsed.

In order to test the quality of flask no. 5, which had been used in the preceding experiment with seed not the freshest, we set up another experiment in the same way as the preceding, using another variety of wheat, no. 1746, of which we had material from 1907 to 1911, inclusive, and putting the freshest and therefore presumably the best in this flask. As before, the temperature in the flask was lower throughout this experiment than in the others. From this we can only infer the inferior quality of this particular flask. The data are given in table XX. Except for the behavior of this particular flask, this experiment conforms to the rule which the preceding tests have indicated, namely that the freshest seeds are also the most vigorous. This is confirmed still further by the germinations in the seed bed.

TABLE XX

Date	Time	Room	1907	1908	1909	1910	1911
February 15..	12:00 P.M.	17°5 C.	16°8 C.	16°8 C.	16°8 C.	16°8 C.	16°4 C.
15..	4:00 P.M.	17.1	16.9	16.9	16.9	17.0	16.5
16..	9:00 A.M.	17.1	17.0	17.1	17.2	17.3	16.7
16..	5:00 P.M.	17.1	17.1	17.2	17.3	17.5	16.8
17..	8:30 A.M.	17.1	17.3	17.3	17.6	17.9	17.0
17..	12:00 M.	17.1	17.3	17.3	17.65	18.0	17.05
17..	4:30 P.M.	17.1	17.4	17.4	17.7	18.1	17.1
18..	8:30 A.M.	17.1	17.5	17.5	18.1	18.7	17.4
18..	4:00 P.M.	17.1	17.6	17.55	18.3	19.0	17.6
19..	8:30 A.M.	17.1	17.7	17.7	18.8	19.7	18.0
19..	4:30 P.M.	17.1	17.7	17.8	19.0	20.0	18.2
20..	9:00 A.M.	17.1	17.75	18.0	19.5	20.4	18.7
20..	6:30 P.M.	17.1	17.8	18.2	19.7	20.4	18.8
21..	8:30 A.M.	17.1	17.8	18.4	19.8	20.4	18.8
21..	4:30 P.M.	17.8	18.4	18.4	19.8	20.4	18.8
22..	9:45 A.M.	17.1	17.7	18.5	19.8	20.2	18.8
Increase in temperature between first and highest readings			1°0	1°7	3°0	3°6	2°4

A series of seeds, no. 639, also including the crops of 5 years, but in flasks of uniformly better quality, is reported upon in table XXI,

the quantities and the preliminary treatment of the seed being the same as before.

TABLE XXI

Date	Time	Room	1907	1908	1909	1910	1911
March 11.....	8:30 A.M.	17°.0 C.	17°.2 C.	17°.2 C.	17°.1 C.	17°.1 C.	17°.4 C.
11.....	3:00 P.M.	16.7	17.3	17.4	17.4	17.4	17.4
12.....	8:30 A.M.	16.5	17.4	17.5	17.7	18.1	18.2
12.....	4:30 P.M.	16.55	17.5	17.6	17.9	18.5	18.6
13.....	8:15 A.M.	16.5	17.7	17.8	18.3	19.85	19.9
13.....	4:30 P.M.	16.5	17.9	18.1	18.6	20.8	20.9
14.....	8:30 A.M.	16.5	18.2	18.8	19.6	21.3	22.5
14.....	4:30 P.M.	16.5	18.4	19.0	20.0	21.1	22.7
16.....	9:00 A.M.	16.5	18.6	19.7	20.3	20.3	21.2
17.....	9:00 A.M.	16.5	18.6	20.1	20.2	20.5	21.0
Increase in temperature between first and highest readings.....			1°.4	2°.9	3°.2	4°.2	5°.1

The high room temperature at the beginning of this test was undoubtedly due to the presence of the experimenter and to the electric light (32 candle-power, carbon filament) which was in use for some little time.

For the sake of completeness, we include also table XXII, showing the behavior of wheat no. 114, of the crops of 1907, 1909, 1910, 1911, under conditions similar to those of the foregoing tests.

TABLE XXII

Date	Time	Room	1907	1909	1910	1911
March 18.....	11:00 A.M.	17°.2 C.	17°.05C.	17°.1 C.	17°.1 C.	17°.1 C.
18.....	4:30 P.M.	16.5	17.1	17.1	17.2	17.1
19.....	9:00 A.M.	16.5	17.2	17.2	17.7	17.4
19.....	6:00 P.M.	16.5	17.3	17.3	18.0	17.6
20.....	9:00 A.M.	16.5	17.5	17.6	18.3	17.95
20.....	4:30 P.M.	16.4	17.6	17.7	18.4	18.2
21.....	9:00 A.M.	16.4	17.9	17.7	18.5	18.5
21.....	4:30 P.M.	16.4	17.9	17.7	18.5	18.5
22.....	10:30 A.M.	16.4	18.1	17.8	18.35	18.4
22.....	4:00 P.M.	16.3	18.2	17.8	18.3	18.4
23.....	11:00 A.M.	16.3	18.3	17.8	18.2	18.2
24.....	10:00 A.M.	16.2	18.5	17.6	17.9	17.9
Increase in temperature between first and highest readings.....			1°.45	0°.7	1°.4	1°.4

For a comparison of these respiration temperatures with the actual percentages of germination under the usual conditions, we

selected 50 good kernels of the crop of each year, from 1907 to 1911, inclusive, of the different varieties which had been tested in Dewar flasks, as the preceding records show. These seeds were quickly washed in a saturated aqueous solution of corrosive sublimate and rinsed four times in boiled distilled water. They were then soaked for 24 hours in distilled water and planted, on January 16 and 17, 1913, in shallow boxes of sand. These boxes contained sand and had previously been steamed for three hours in an Arnold steam sterilizer and thereupon allowed to cool. Pending the germination of the seeds, these boxes stood on the benches in the greenhouse. In the last four columns to the right, in table XXIII, will be found the measurements and the percentage of growth of the plumules of the seedlings from the seeds of different ages. These percentages were obtained by using the longest plumules as an arbitrary standard for comparison. In addition, therefore, to the information regarding actual germination which this table gives, we have also a record of the amounts of growth in length of these seedlings. These figures show that the older seed is not only slower in germinating and that there are fewer germinating seeds the older the seed is, but also that the seedlings, when the seed does germinate, are inferior in size to those from fresher seed, even when grown under the same conditions. The temperatures developed, therefore, within a short time in such an excellent insulator as a silvered Dewar flask, indicate plainly the values of the lots of seeds sampled. It would have been interesting for us to follow the later development of these seedlings of different ages, and to compare the harvests from the different lots, but the conditions of our experiments were such that this was not at the time possible. Table XXIII, supplementing the figures of previous tables showing the results of our Dewar flask experiments, shows the percentages of germination and also the percentages of growth immediately following germination.

Inspection of table XXIII shows an almost surprisingly regular decrease in the number of seeds sprouting as the age of the seed increases. This is plainest in the varieties numbered 791 and 98. The reasons for the less regularity in the other three varieties are not known to us. We have already pointed out that the different

flasks are not equally perfect insulators, and that the various conditions of harvesting and storage would also affect the results. Nevertheless, it is plain that the evidence of Dewar flask and of seed bed are quite consistent. Germination is increasingly tardy

TABLE XXIII

Wheat	Year	No. of kernels	No. sprouting January		Per cent-age spr. Jan. 27	Lengths of plumules in cm. January 27–28			
			25	27		Short-est	Long-est	Average	Percent-age of growth
Talaverra spring..	1871	50	.....	.....	0	.....	.....	.....	.....
California white spring.....	1872	50	.....	.....	0	.....	.....	.....	.....
No. 791.....	1909	50	45	46	92	0.4	7.0	4.75	74
	1910	50	47	47	94	1.0	8.1	5.67	88
	1911	50	47	49	98	1.1	8.5	5.95	92
No. 98.....	1909	50	28	35	70	0.3	6.8	3.73	58
	1910	50	33	36	72	0.2	7.8	4.28	66
	1911	50	34	41	82	1.0	7.1	4.63	72
	1876	50	.....	.....	Jan. 30 0	.....	.....	.....	.....
No. 1746.....	1907	50	27	29	58	0.7	7.3	3.62	56
	1908	50	6	6	12	1.4	8.8	6.41	100
	1909	50	36	45	90	0.3	8.5	3.46	53
	1910	50	36	38	76	0.3	8.1	2.81	43
	1911	50	39	43	86	1.3	8.5	5.35	83
No. 639.....	1907	50	26	26	52	0.2	6.2	2.66	32
	1908	50	15	18	36	0.2	3.4	1.20	18
	1909	50	26	29	58	0.3	6.5	2.49	38
	1910	50	41	42	84	0.4	7.3	2.71	42
	1911	50	50	50	100	0.2	7.4	2.41	37
No. 114.....	1907	50	34	36	72	0.2	6.2	2.75	42
	1909	50	43	44	88	0.6	6.9	3.84	59
	1910	50	42	44	88	1.0	6.0	2.94	45
	1911	50	44	44	88	0.3	8.2	3.32	51
	1876	50	.....	.....	0	.....	.....	.....	.....
	1876	50	.....	.....	0	.....	.....	.....	.....
	1876	50	.....	.....	0	.....	.....	.....	.....
Central Kansas I	1877	50	0	0	0	0	0	0	0
Central Kansas II	1877	50	0	0	0	0	0	0	0
Iowa white winter	1887	50	.....	.....	0	.....	.....	.....	.....

the older the seed. Furthermore, the percentages of growth, as shown by the lengths of the plumule, accord perfectly with the percentages of germination and correspond with the temperatures attained in the flasks. In varieties numbered 791 and 98, for example, there is the same regular decrease in growth as in

germination with the increasing age of the seed. The younger and more viable seeds sprout more promptly and produce a greater

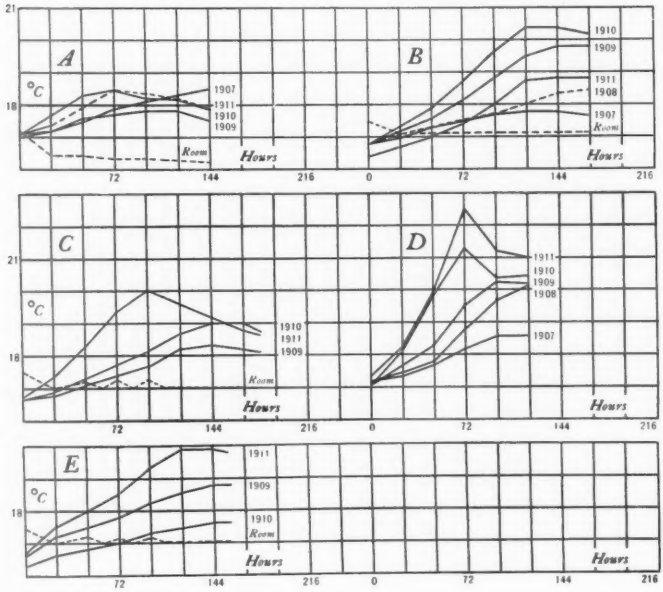


FIG. 15.—Wheat in flasks: *A*, no. 114; *B*, no. 1746; *C*, no. 791; *D*, no. 639; *E*, no. 98.

early growth than the older ones. The accompanying graphs (figs. 15–17) facilitate a comparison of the results as indicated in the foregoing tables.

Summary

Our experiments with seeds of different known ages indicate that one may readily ascertain the quality of these seeds, that is, their germinating power or viability, and the vigor of their growth immediately following germination, by determining the temperatures which they will develop in silvered Dewar flasks under conditions suitable for germination.

Each species of plant which we have studied appears to have, like the higher animals, a "normal" temperature, departures from

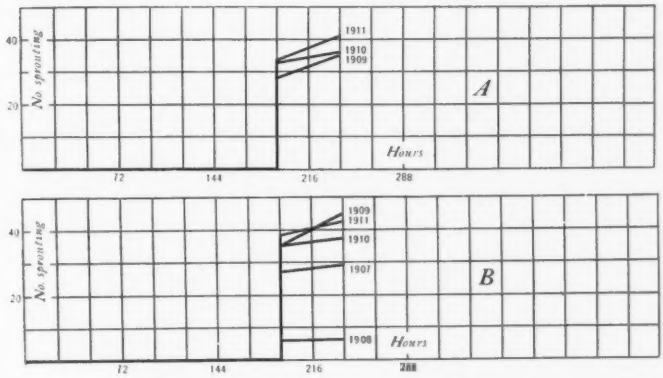


FIG. 16.—Wheat in soil: *A*, no. 98; *B*, no. 1746

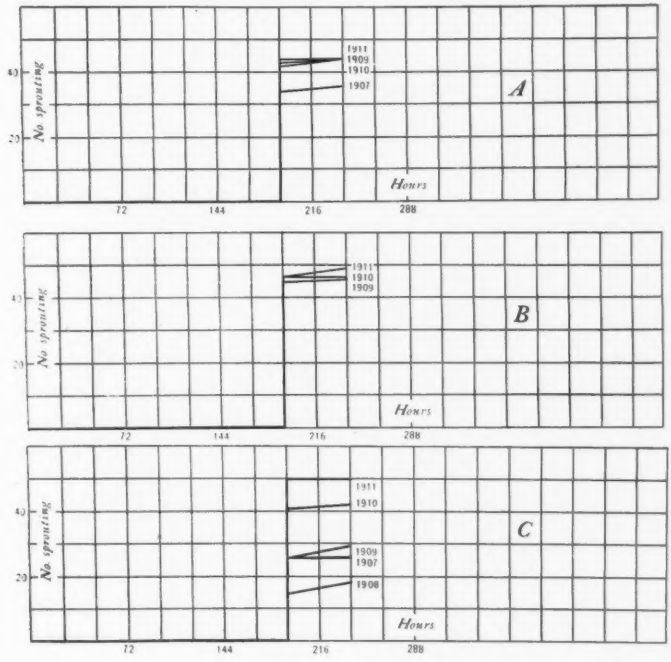


FIG. 17.—Wheat in soil: *A*, no. 114; *B*, no. 791; *C*, no. 639

which indicate departures from the best condition of the organism. A temperature in excess of the normal generally indicates an infection; a subnormal temperature, on the other hand, indicates lessened vigor. Decreased vigor is very generally due to increased age.

This "normal temperature" has been worked out graphically for some of our seeds and is shown in fig. 18, in which is indicated the average daily heat yield, in terms of 10 grams of seed of different sorts, all of them from the crop of 1911 and experimented with in the academic year 1911–1912. Inspection of the figure

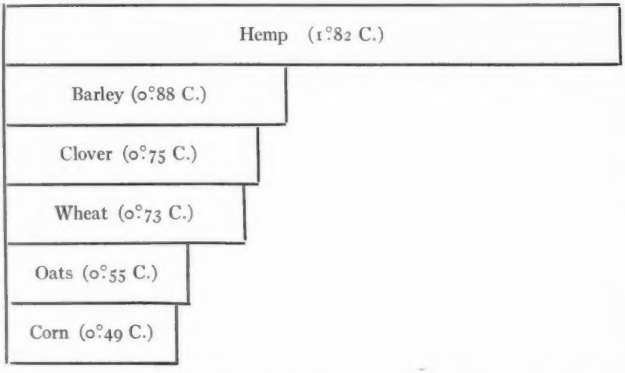


FIG. 18.—Showing the average daily heat yield in terms of 10 grams; 1911 seed

shows, for example, that a temperature curve indicating normal germinating power in oats would mean less than 50 per cent germination if given by an equal quantity of barley.

Departures from the "normal" temperatures are accompanied by differences in the amounts of growth immediately following germination. This may be true of the other stages in the life of the plant, as is the case in the higher animals, though the nature of our experiments does no more than suggest this possibility.

LELAND STANFORD JUNIOR UNIVERSITY
STANFORD UNIVERSITY, CALIFORNIA

THE DEVELOPMENT OF THE EMBRYO SAC IN THE CONVALLARIACEAE

F. McALLISTER

(WITH PLATES VI AND VII)

The family Convallariaceae as used by BRITTON (2) is the equivalent of the family Asparagoideae as it is used by ENGLER and PRANTL (12). GRAY'S *New manual* (20) has not made use of either of the above family names, but has distributed the 10 genera occurring within the family in the tribes Polygonateae and Parideae. In this paper I shall refer to this group of genera as the Convallariaceae.

Of the 10 genera of the Convallariaceae occurring in the northeastern part of the United States, *Clintonia* has been investigated by SMITH (23), *Convallaria* by WIEGAND (24), *Trillium grandiflorum* by ERNST (13), and *T. recurvatum* by COULTER and CHAMBERLAIN (11). I have in earlier papers reported on *Smilacina stellata* (18) and *S. racemosa* (19), and in this paper report my investigations on *Maianthemum*, *Streptopus*, *Polygonatum*, and *Medeola*. The only genera occurring within this range which have thus far not been reported upon are *Asparagus* and *Disporum*.

Much of this work on the Convallariaceae was carried on at Beloit College during the years 1907 and 1908. Material for the study of *Smilacina stellata*, *S. racemosa*, *Polygonatum commutatum*, and *Maianthemum canadense* were collected in the vicinity of Beloit, Wisconsin. Professor W. J. V. OSTERHOUT kindly supplied me with fixed material of *Smilacina sessifolia* from the vicinity of Berkeley, California, and Professor H. D. DENSMORE very generously turned over to me considerable material of *Smilacina amplexicaulis* which he had collected and fixed in California. I am indebted to Mr. R. E. WEBSTER for material of *Clintonia borealis* from the vicinity of Iron Mountain, Michigan. Flowers of *Streptopus roseus* and *Medeola virginica* were collected and fixed in the vicinity of Ithaca, New York, in the spring of 1912.

The publication of these results has been delayed from time to time, partly owing to the press of other work and partly through the hope of including other members of the group in this report.

I have reviewed in another connection (**18**) the scanty literature on the development of the embryo sac among members of the Convallariaceae. Two accounts of the development of the embryo sac of *Convallaria majalis* differ radically as to the fate of the first four nuclei resulting from the division of the nucleus of the mother cell. According to WIEGAND'S account (**24**), all 4 of these nuclei enter into the structure of the mature embryo sac, while SCHNIEWIND-THIES (**21**) reports that but one of 4 megaspores enters into the structure of the embryo sac.

ERNST (**13**) reports that in *Trillium grandiflorum* the inner of the 2 cells resulting from the heterotypic division develops into the embryo sac, while CHAMBERLAIN (**9**) finds that in *Trillium recurvatum* the inner of 4 megaspores develops into the embryo sac. ERNST (**13**) also found that in *Paris quadrifolia* the inner of the first 2 cells arising from the first division of the mother cell formed the mature embryo sac by three divisions of its nucleus, as was the case in *Trillium grandiflorum*.

In the paper referred to above (**18**) I have shown that in *Smilacina stellata* the megaspore mother cell divides to form 4 fully separated megaspores, and that the separating membranes later disappear, forming thus a tetranucleate cell, from which, by one division of its 4 nuclei, the mature embryo sac is formed.

Since the publication of the above-mentioned paper, one other member of the family has been reported upon. According to SMITH (**23**), the reduction divisions of the megaspore mother cell of *Clintonia borealis* result in the formation of a tetranucleate cell, the outermost nucleus of which is plump and normal, while the remaining 3 inner nuclei are shrunken and disorganized. The outermost nucleus divides twice, and 3 of the resulting 4 nuclei organize an apparently normal egg apparatus, while the remaining nucleus serves as the single polar nucleus. I have been able to confirm SMITH'S results from material collected in the vicinity of Iron Mountain, Michigan, a few sections of which I had already prepared at the time of the publication of his results.

My work on the embryo sac of *Smilacina racemosa* (**19**) has shown that in closely related species of the same genus a marked difference may exist in the origin of the nuclei entering into the structure of the embryo sac. In *S. racemosa* the mother cell divides unequally, forming a large outer and a small inner cell. Later stages show the division membrane to be permanent. From the outer cell the 8 nuclei of the mature embryo sac are formed by three divisions. Split cell membranes separate for a short time the two pairs of nuclei resulting from the division of the heterotypic daughter nuclei (figs. 28, 29). The nucleus of the small inner cell divides but once, and the 2 resulting nuclei gradually degenerate. The embryo sac in *S. racemosa* is thus formed from the 2 outer of the 4 potential megaspores. *S. stellata* (**18**), on the other hand, forms its embryo sac from 4 megaspores which become merged in one 4-nucleate cell by the disappearance of the partition membranes.

Smilacina sessifolia

As cited above, the embryo sac of *S. stellata* is formed from 4 fully separated megaspores which become merged in one cell, one further division of the 4 nuclei forming the 8-nucleate embryo sac (figs. 26, 27). Three divisions of the nucleus of the megaspore mother cell thus produce the necessary number of nuclei for the mature embryo sac, as in cases of the so-called *Lilium* type of embryo sac formation.

I have found that in *S. sessifolia* the 4 nuclei resulting from the first two divisions of the megaspore mother cell are at first separated by cell membranes, and later, owing to the disappearance of the membranes, come to occupy a large common cell precisely as in *S. stellata*.

Fig. 3 shows the 4 cells arranged bilaterally, while in fig. 4 a linear row of 4 is to be seen. The bilateral arrangement here is much the more abundant. The cell membranes of the second division, as is the case in *S. stellata*, are the first to disappear (fig. 5), forming thus 2 binucleate cells. Later the middle membrane disappears, giving rise to a tetranucleate cell (fig. 6). This

cell enlarges rapidly by vacuolization (fig. 7) and its nuclei undergo another division to form the 8-nucleate embryo sac (fig. 8).

Smilacina amplexicaulis

I have also in an earlier paper (19) referred to the apparent identity of the embryo sac development in *S. racemosa* with that of *S. amplexicaulis*. A more careful examination of the first two divisions of the megaspore mother cell of the latter species confirms these earlier observations.

The megaspore mother cell of *S. amplexicaulis* may be hypodermal (fig. 11), or it may be one or two cell layers beneath the epidermis. Fig. 13 illustrates the unequal first division of the mother cell to form a large outer cell and a small inner one. A cell plate is formed which splits, causing the complete separation of these 2 cells. Later phases show this separation to be permanent. These heterotypic daughter nuclei divide simultaneously, and conspicuous cell plates are formed (figs. 14, 15), but I have never observed the splitting of the membranes as is the case in *S. racemosa*. In the latter species the complete separation of these homoeotypic nuclei seemed to be of short duration, and it is probable that more abundant material would show that in the case of *S. amplexicaulis* complete membranes are also formed, which, however, disappear early to form the 2 unequal binucleate cells (fig. 16).

Because of scarcity of material, I have been unable to follow the development of this embryo sac farther than the 4-celled stage. The close similarity of the two species (*S. amplexicaulis* being the Pacific Coast form of *S. racemosa*) makes it very probable that the embryo sac development is identical in the two species.

Maianthemum canadense

In *Maianthemum canadense* the first division of the nucleus of the megaspore mother cell is followed by the formation of a cell membrane which separates the 2 resulting nuclei (fig. 19). Similar membranes are formed separating the homoeotypic nuclei, so that 4 fully separated cells result (figs. 20, 21), as is the case in *Smilacina stellata* and *S. sessifolia*. As is shown in figs. 19–21, the 4 resulting megaspores are rarely arranged in an axial row, but

are usually arranged more or less bilaterally. Although I was not able to identify split cell membranes between these reduction nuclei, the cell plates are nevertheless very different from "evanescent cell plates" such as are to be seen, for example, in the first divisions in the developing embryo sac of *Lilium*. In *Maianthemum* the cell membrane extends out and joins with the lateral wall of the mother cell, while all traces of the central spindle disappear. Later the partition membranes degenerate, thus giving rise to a tetranucleate cell (fig. 22). This tetranucleate cell enlarges by the formation of vacuoles in its interior (figs. 23, 24), and its nuclei undergo a further division to form the 8-nucleate embryo sac (fig. 25).

Streptopus roseus

The first division of the megaspore mother cell of *Streptopus roseus* results in the formation of 2 equal and fully separated daughter cells (fig. 30). The inner cell enlarges, gradually compressing the outer into a disorganized crescent-shaped mass (fig. 31).

Before the outer cell has been completely disorganized, the inner daughter cell divides, forming, in some cases at least, 2 fully separated daughter cells (fig. 32). In most cases, however, the partition wall is lacking, either not having been formed or having degenerated after formation (fig. 35). It is of course impossible to determine whether a partition wall is formed in all cases, but since the evidence is clear that it is frequently formed, it seems reasonable to expect that it is formed in all cases and subsequently undergoes degeneration.

Widely separated daughter cells such as are illustrated in fig. 33 are occasionally to be seen. While this wide cleft between the cells is in this case, without doubt, due to plasmolysis, such a wide separation could of course not have taken place had not the 2 cells been perfectly distinct. Whether 2 such widely separated cells can fuse to form a single binucleate cell may be doubted. Further evidence on this point is to be seen in fig. 34. Here the nuclei of 2 homoeotypic daughter cells have each divided, forming 2 distinct binucleate cells. The disorganized mass at the micropylar end of the outer cell is the remains of the outer cell of the first division. A tendency is thus shown toward the formation of an embryo sac

from each of the potential megaspores arising from the inner heterotypic daughter cell.

The binucleate cell arising from the homoeotypic division in the inner (heterotypic) daughter cell enlarges greatly by vacuolization, and by two further nuclear divisions forms the 8-nucleate embryo sac (figs. 36, 37). After a period of growth, a typical egg apparatus and an antipodal group are organized. The polar nuclei fuse and the fusion nucleus takes up a position close to the egg.

Medeola virginica

The very large megaspore mother cell of *Medeola virginica* is in many cases hypodermal. Fig. 38 shows a very common condition in which the mother cell in synapsis lies in contact with the epidermis.

Although a conspicuous cell plate is formed at the time of the first division of the nucleus of the mother cell (fig. 39), it does not, so far as I can determine, result in the formation of a division membrane. It entirely disappears before the homoeotypic division.

The outer heterotypic nucleus divides slightly in advance of the inner, and temporary cell plates are formed between each pair of nuclei (fig. 40). These cell plates persist for some time, often being visible after the third division of the mother cell (fig. 43).

The daughter nuclei resulting from the division of the inner heterotypic nucleus may be equal in size (fig. 40) or they may be very unequal (fig. 41), the innermost nucleus being much smaller than the outer. In several cases, in fact, the inner nucleus appeared so shrunken and disorganized as to be very probably incapable of further division (fig. 42). Such a condition would cause embryo sacs with but 2 antipodal cells, and it seems very probable that this is occasionally the case.

Frequently the small inner nucleus divides at the time of the division of its larger sister nucleus, the result being 2 large and 2 small nuclei. One of the large nuclei is a polar nucleus, and the other, with the 2 small nuclei, forms the 3 antipodal nuclei (fig. 43). In still other cases, where the division of the inner heterotypic nucleus has been such as to form 2 nuclei of equal size, 3 uniform antipodal nuclei are formed (fig. 44).

A normal egg apparatus is formed from the 3 micropylar nuclei. In other respects as well the embryo sac seems normal.

Polygonatum commutatum

In *Polygonatum commutatum* the megaspore mother cell is frequently hypodermal (fig. 45), or it may be separated from the epidermis by one or two layers of cells (fig. 46). The mother cell divides twice to form an axial row of 4 permanently separated megaspores (figs. 47, 48). The inner spore enlarges by vacuolization, while the outer 3 gradually shrivel and disorganize (fig. 49). Three divisions of the nucleus of this functional megaspore form the 8 nuclei of the mature embryo sac (figs. 50, 51, 52). A normal embryo sac is organized from the 8 nuclei thus formed. The 2 polar nuclei fuse early, and the fusion nucleus lies in close proximity to the antipodal cells until immediately before fertilization, when it takes up a position near the egg.

This mode of embryo sac development has been called the "normal" as contrasted with the "lily type" of development. It seems clear, especially from the embryo sac behavior in the Convallariaceae, that no one type of development can be regarded as "normal."

I have shown that in the cases of *Smilacina stellata* and *S. racemosa* (**18, 19**) reduction in the number of the chromosomes takes place with the first two divisions of the megaspore mother cells. While I have been unable to make reliable countings of the chromosomes in the case of the other species studied, I have found evidences of the reduction divisions, such as the synapsis stage and double heterotypic chromosomes, in all the forms investigated, with the exception of *Streptopus roseus*, in which I was unfortunately unable to get the first division of the mother cell. In view of the indirect evidence mentioned above, it seems safe to conclude that, as with *Smilacina stellata* and *S. racemosa*, reduction in the chromosome number accompanies the first two divisions of the mother cell in the members of the group which I have studied. In *Clintonia* (**23**), *Trillium* (**11, 13**), and *Paris* (**13**), the evidence shows clearly that the first two divisions of the megaspore mother cells are reduction divisions.

Two megaspore mother cells

Among the monocotyledonous plants, 2 megaspore mother cells in the same nucellus have been reported in but few instances. In *Ornithogalum pyrenacium*, GUIGNARD (**15**) reports that 2 large cells ("deux grosses cellules collaterales") are often present at the apex of the nucellus. BERNARD (**1**) has reported 2 embryo sacs in *Lilium candidum* in the same nucellus. COULTER and CHAMBERLAIN (**11**) report two cases in *Lilium philadelphicum*, in one of which 3 "archesporial cells" were present in the same nucellus and in the other 5. Miss FERGUSON (**14**) has called attention to a single case of the occurrence of 2 mother cells separated by a layer of somatic cells in a single megasporangium of *Lilium longiflorum*. LECHMERE has also reported (**16**) for *Fritillaria messanensis* 2 embryo sacs which apparently have arisen from distinct mother cells side by side in a single ovule.

In the genus *Smilacina* 2 megaspore mother cells or their derivatives occur occasionally in the same nucellus. In *S. stellata* I have observed in four different cases 2 partially developed embryo sacs in the same nucellus, separated by somatic tissue (fig. 10). There can be no doubt that these have arisen from distinct mother cells. In *S. sessifolia* 2 mother cells were frequent. On one raceme, approximately one nucellus out of 4 contained either 2 mother cells or 2 developing embryo sacs. As is shown in fig. 9, they are usually separated more or less by somatic cells. In *S. racemosa* 2 mother cells or embryo sacs were in several cases observed lying side by side in the same nucellus, in some instances lying in contact and in others separated by sterile cells. In *S. amplexicaulis* 2 mother cells in the same ovule were observed on several occasions. While a complete record was not kept of all preparations showing this, I have records of five such abnormalities. As is shown in fig. 12, they are usually separated more or less by somatic cells.

In *Polygonatum commutatum* 2 mother cells in one megasporangium were occasionally observed (fig. 45). Four such cases have been recorded but others were observed. In *Maianthemum canadense* three cases were also observed of 2 megaspore mother cells in the same nucellus. As will be seen from fig. 17, the nucellus is abnormally broad and the mother cells are partially separated by disintegrating somatic cells.

It is probable that little significance is to be attached to these abnormalities. The occurrence of more than one megaspore mother cell in a nucellus is common in the dicotyledonous plants, and it has been rather remarkable that more have not been found among the monocotyledons.

As will be seen from figs. 11, 12, 17, and 18, the ovules in which 2 mother cells or 2 embryo sacs have been figured are all conspicuously broader than those containing but one mother cell. The relative proportion of the sterile tissue in the two cases is essentially the same, thus suggesting that where a normal amount of sterile tissue is present but one mother cell is formed.

Discussion

It will be seen from the foregoing that within the limits of the Convallariaceae considerable variation exists as to the origin of the embryo sac. In *Polygonatum commutatum* and in *Trillium recurvatum* (**9**) the embryo sac arises from one of an axial row of 4 megaspores. In *Clintonia borealis* (**23**) it arises from the outer of 4 megaspore nuclei, which are, however, not separated by cell walls or membranes. In *Smilacina racemosa* and in *S. amplexicaulis* it has its origin from the outer daughter cell of the first division of the mother cell; while in *Streptopus roseus*, *Trillium grandiflorum* (**13**), and *Paris quadrifolia* (**13**) the embryo sac arises from the corresponding inner daughter cell. In *Smilacina stellata*, *S. sessifolia*, and *Maianthemum canadense* 4 fully separated megaspores, by the disappearance of the partition walls, become merged in one tetranucleate cell. One division of each of these 4 nuclei forms the 8 nuclei of the complete embryo sac. The immature embryo sac of *Convallaria majalis*, according to WIEGAND (**24**), has a single cell membrane separating the inner pair of reduction nuclei from the outer pair, and in *Medeola virginica* a pronounced cell plate is developed between the daughter nuclei of the first division of the mother cell, which, however, disappears before the second division, and definite though temporary cell plates are also formed between the homoeotypic nuclei. Thus it will be seen that in this group, one, two, or four of the reduction nuclei may enter into the structure of the mature embryo sac.

In those forms in which more than one reduction nucleus enters into the structure of the embryo sac, we find various degrees of separation of these nuclei in temporary cells. In *Smilacina stellata* and *S. sessifolia* and in *Maianthemum canadense* the 4 reduction nuclei become fully separated by cell membranes, which later disappear. In *Smilacina racemosa*, whose embryo sac arises from the outer of 2 daughter cells, the homoeotypic nuclei are for a time fully separated from one another by split cell plates. In *S. amplexicaulis* definite cell plates are present between these homoeotypic nuclei, and it seems probable that an examination of sufficient material would show the splitting of these cell plates. In *Streptopus roseus*, whose embryo sac arises from the inner daughter cell of the first division, a definite cell plate is formed which, at times at least, splits, thus causing complete separation of the 2 homoeotypic nuclei. The single cell membrane in the developing embryo sac of *Convallaria majalis* (**24**) and the cell plates in *Medeola* show the tendency here toward the separation of the products of the reduction divisions into distinct cells.

There seems to be substantial agreement that in cases where one cell of an axial row of 4 develops into the embryo sac, the 4 cells are to be regarded as megaspores, and the cell from which these have arisen a megaspore mother cell. Both megaspore mother cell and megaspore are regarded as the morphological equivalents of the microspore mother cell and microspore respectively. In those cases, however, in which the embryo sac develops from one of the 2 daughter cells of the megaspore mother cell or from the undivided megaspore mother cell, in general two opposing views are prevalent. The one, elaborated by COULTER (**10**), regards reduction as a criterion by which spores or spore nuclei may be identified, and holds that even though the reduction nuclei are not separated they should nevertheless be regarded as megaspore nuclei. The other view, probably most actively supported by CAMPBELL (**6, 7**) and BROWN (**3, 4**), holds that reduction is not the "sole criterion" for distinguishing spores and gametophytes, and that the cell giving rise to the embryo sac should be regarded as a megaspore, whether it is the undivided megaspore mother cell, one of 2 daughter cells, or one of 4 daughter cells.

It seems clear that these two views are due to a difference in the usage of the terms "megaspore mother cell" and "megaspore," rather than to any misunderstanding or misinterpretation of the data. That the megaspore mother cell of *Lilium* has a function similar to that of the inner megaspore of the row of 4 in *Polygonatum* may be the case, inasmuch as both give rise to the embryo sac, but the mother cell in the first case has arisen from the tissue of the nucellus, in the same position and by almost identical cell divisions as in *Polygonatum*. Aside even from any consideration of the reduction phenomena, there would seem to be no doubt as to the strict homology of the megaspore mother cells in these two forms on the basis of their position in the nucellus, their conspicuous size, the character of their protoplasm, and the mode of their differentiation from the tissue of the nucellus. This conclusion applies equally well to the megaspore mother cells of most other angiosperms. The fact that the heterotypic division occurs in the megaspore mother cell of *Lilium* may be regarded as confirmatory evidence of its nature.

The first 4 nuclei of the lily embryo sac originate, also, by steps identical with those giving rise to the nuclei of the megaspores of *Polygonatum*. These 4 nuclei in the above-mentioned two forms are homologous, whether we call them "megaspore nuclei" or "the first 4 nuclei of the embryo sac." The increasing number of cases in which the first 4 nuclei arising from the megaspore mother cell are found to be more or less separated by temporary cell membranes suggests very strongly that the conditions in *Lilium* have arisen in a like manner, namely the loss by the 4 megaspores of their physiological individuality as spores and the subsequent disappearance of the separating membranes to form the 4-nucleate stage of the embryo sac.

In *Smilacina stellata* (**18**) I have shown that the 4 reduction nuclei are completely separated, the cell plate having split so that wide clefts between the cells are often present. This separation is due probably to shrinkage in fixation. The cell membranes cannot be regarded in the same light as evanescent cell plates, for here the cell plates are completely formed, after which they split to form the distinct membranes of the new cells. There can be no

doubt as to the individuality of the 4 cells, and that they are the morphological equivalents of megaspores is beyond question.

In *Smilacina racemosa* (19), although but the 2 outer reduction nuclei enter into the structure of the embryo sac, they too are for a time fully separated by a split cell plate.

In *Streptopus roseus*, also, the cell plate which separates the 2 homoeotypic nuclei entering into the structure of the embryo sac very often splits, thus causing a complete separation of these 2 nuclei. I have referred above to evidence which suggests that at times this separation may be permanent.

No other conclusion seems possible than that 4 fully separated cells arising from a megaspore mother cell by the reduction divisions must be megaspores. That the 2 cells which in *Smilacina racemosa* and in *Streptopus roseus* fuse to form the 2-celled stage of the embryo sac are also megaspores seems equally clear.

The cell membranes separating the reduction nuclei in the megasporangia of *Smilacina sessifolia*, *S. amplexicaulis*, and *Maianthemum canadense*, although in my material showing no splitting into two distinct cell walls, are nevertheless much more complete and persistent than the evanescent cell plates such as have been reported at times for the embryo sac of *Lilium* and in other genera. These membranes extend out and join the lateral walls and all traces of a central spindle disappear. It seems very possible that an examination of sufficient stages would show that these membranes also split as is the case in *Smilacina stellata*.

The transition from forms like the foregoing, in which the division membranes persist for some time, to the lily type of embryo sac is strongly suggested by those forms in which the membranes are incomplete and evanescent, as is the case in *Medeola* and *Clintonia*. Additional evidence on this point has been contributed by BROWN and SHARP for *Epipactis* (5). According to their account, although the embryo sac usually arises from the inner of an axial row of 4 megaspores, it also frequently arises from the megaspore mother cell, evanescent cell membranes being formed, and it may possibly be formed from the 2 inner megaspores. Evidence on this latter point, however, was not conclusive.

The cell plates or membranes in the embryo sac of *Medeola* and of *Epipactis* are simply more fully formed and more persistent than those of *Lilium*, and a little less fully formed and less enduring than those of *Smilacina* and *Maianthemum*. The question as to the relative completeness and duration of the membranes separating the reduction nuclei in these forms is one that cannot affect the homology of the cells in question. They are all morphologically megaspores. That those reduction nuclei which are not even separated by evanescent cell plates, if such exist, are also to be regarded as megaspore nuclei would seem, on the basis of their homology with the above-mentioned forms, to be an unavoidable conclusion.

The presence or absence of temporary or evanescent cell plates between the reduction nuclei as a means of determining whether the mother cell is to be regarded as a megaspore or whether each of the reduction nuclei is to be regarded as the nucleus of a megaspore (**4**) seems in the light of the above evidence to be not only "inconclusive" but worthless. I have shown that the presence even of split cell membranes is no indication that such membranes will be permanent, while, on the other hand, the lack of division membranes between the reduction nuclei does not necessarily result in the lily type of embryo sac formation. In the cases of *Eichhornia* (**22**), *Avena* (**8**), *Crucianella* (**17**), and *Asperula* (**17**), although division membranes are lacking, nevertheless the functional megaspores develop exactly as though such membranes were present. In *Crucianella* all 4 megaspores may germinate, though ultimately but one embryo sac matures while the other 3 degenerate. If the lack of cell plates or membranes between the reduction nuclei really signified that the reduction divisions have been shifted so that they occur in the embryo sac, we should have upon the division of the homoeotypic nuclei in *Crucianella* an 8-nucleate embryo sac. Subsequent development shows, however, that we really have 4 embryo sacs "in tandem."

Summary

1. In the eight members of the Convallariaceae investigated by the author, the embryo sac is formed from one reduction nucleus

in *Polygonatum*, from 2 in *Smilacina racemosa*, *S. amplexicaulis*, and *Streptopus roseus*, and from 4 in *Smilacina stellata*, *S. sessifolia*, *Maianthemum canadense*, and *Medeola virginica*.

2. In all forms in which more than one reduction nucleus enters into the structure of the embryo sac, these nuclei are at first more or less completely separated by cell membranes, the degree of separation varying from split cell plates in *Smilacina stellata* to evanescent cell plates in *Medeola virginica*.

3. The difference in the degree of the separation of these cells cannot affect their morphological status; they are all megaspores.

4. In the light of this evidence it seems reasonable to conclude that all reduction nuclei arising from the nucleus of the megaspore mother cell, whether temporarily separated or not separated at all, should be regarded as megaspore nuclei.

5. Two megaspore mother cells were occasionally observed in six of the eight species investigated.

UNIVERSITY OF TEXAS
AUSTIN, TEXAS

LITERATURE CITED

1. BERNARD, C. H., Recherches sur les sphères attractives chez *Lilium candidum*, etc. Jour. Botanique **14**:118–124, 177–188, 206–212. 1900.
2. BRITTON, NATHANIEL LORD, Manual of the flora of the northern states and Canada. New York. 1901.
3. BROWN, WILLIAM H., The nature of the embryo sac of *Peperomia*. BOT. GAZ. **46**:445–460. 1908.
4. ———, The embryo sac of *Habenaria*. BOT. GAZ. **48**:241–250. 1909.
5. BROWN, WILLIAM H., and SHARP, LESTER W., The embryo sac of *Epipactis*. BOT. GAZ. **52**:439–452. 1911.
6. CAMPBELL, D. H., The embryo sac of *Pandanus*. Bull. Torr. Bot. Club **36**:205–220. 1909.
7. ———, The embryo sac of *Pandanus*. Ann. Botany **25**:773–789. 1911.
8. CANNON, W. A., A morphological study of the flower and embryo of the wild oat, *Avena fatua*. Proc. Calif. Acad. Sci. III. **1**:329–364. 1900.
9. CHAMBERLAIN, C. J., Winter characteristics of certain sporangia. BOT. GAZ. **25**:124–128. 1898.
10. COULTER, J. M., Relation of megaspores to embryo sacs in angiosperms. BOT. GAZ. **45**:361–366. 1908.
11. COULTER, J. M., and CHAMBERLAIN, C. J., Morphology of the angiosperms. New York. 1903.

12. ENGLER, A., and PRANTL, K., Die natürlichen Pflanzenfamilien. II. Teil. 5. Abteilung. 1888.
13. ERNST, A., Chromosomenreduktion, Entwicklung des Embryosackes, und Befruchtung bei *Paris quadrifolia* und *Trillium grandiflorum*. Flora **91**:1–46. 1902.
14. FERGUSON, MARGARET C., Two embryo sac mother cells in *Lilium longiflorum*. BOT. GAZ. **43**:418–419. 1907.
15. GUIGNARD, L., Recherches sur le sac embryonnaire des Phanérogames Angiospermes. Ann. Sci. Nat. Bot. VI. **13**:136–199. 1882.
16. LECHMERE, A. E., Two embryo sac mother cells in the ovule of *Fritillaria*. New Phytologist **9**:257–259. 1910.
17. LLOYD, F. E., The comparative embryology of the Rubiaceae. Mem. Torr. Bot. Club **8**:27–112. 1902.
18. McALLISTER, F., The development of the embryo sac of *Smilacina stellata*. BOT. GAZ. **48**:200–215. 1909.
19. ———, On the cytology and embryology of *Smilacina racemosa*. Trans. Wis. Acad. Sci. **17**:599–660. 1913.
20. ROBINSON, B. L., and FERNALD, M. L., Gray's new manual of Botany. Seventh edition. New York. 1908.
21. SCHNIEWIND-THIES, J., Die Reduktion der Chromosomenzahl und die ihr folgenden Kerntheilungen in den Embryosackmutterzellen der Angiospermen. Jena. 1901.
22. SMITH, WILSON R., A contribution to the life history of the Pontederiaceae. BOT. GAZ. **25**:324–337. 1898.
23. ———, The tetranucleate embryo sac of *Clintonia*. BOT. GAZ. **52**:209–217. 1911.
24. WIEGAND, K. M., The development of the embryo sac in some monocotyledonous plants. BOT. GAZ. **30**:25–47. 1900.

EXPLANATION OF PLATES VI and VII

All the figures were made with the aid of a camera lucida and reduced one-third. The magnification (reduced) is about 400, with the exception of fig. 38, which has a magnification of 200. Unless otherwise indicated, the micropyle is down in the figures.

Smilacina sessifolia

FIG. 1.—Embryo sac mother cell recovering from synapsis.

FIG. 2.—First division of megaspore mother cell.

FIG. 3.—Four fully separated megaspores arranged bilaterally.

FIG. 4.—Four fully separated megaspores in an axial row.

FIG. 5.—The membranes of the homoeotypic division have disappeared, leaving only the membrane of the first division.

FIG. 6.—No traces of membranes remain; the 4-nucleate stage of the embryo sac.

FIG. 7.—A slightly older embryo sac.

FIG. 8.—An 8-nucleate embryo sac.

FIG. 9.—Two immature embryo sacs in the same nucellus.

Smilacina stellata

FIG. 10.—Cross-section of nucellus showing 2 megaspore mother cells.

Smilacina amplexicaulis

FIG. 11.—Mother cell hypodermal, in synapsis.

FIG. 12.—Two mother cells in synaptic stage in the same nucellus.

FIG. 13.—The unequal first division of the mother cell.

FIG. 14.—The second division of the mother cell; the cells of the first division completely separated.

FIG. 15.—Second division; cell plates between the homoeotypic nuclei.

FIG. 16.—The 4 reduction nuclei are now contained in 2 binucleate cells; the inner pair of nuclei are degenerating, while the outer pair, from which the embryo sac develops, are plump and normal.

Mianthemum canadense

FIG. 17.—Two megaspore mother cells in synaptic condition.

FIG. 18.—A single megaspore mother cell.

FIG. 19.—The first division of the megaspore mother cell.

FIG. 20.—Four fully separated cells arising from the megaspore mother cell; the arrangement of the 4 cells very irregular.

FIG. 21.—A similar stage, showing again 4 fully separated cells.

FIG. 22.—A later stage in which the cell membranes separating the cells have disappeared, causing the 4-nucleate stage of the embryo sac.

FIGS. 23, 24.—Stages in the vacuolization of the tetranucleate embryo sac.

FIG. 25.—An immature 8-nucleate embryo sac.

Smilacina stellata

FIG. 26.—Four megaspores fully separated by split cell plates.

FIG. 27.—The cell membranes of the second division have disappeared, but the split cell plate of the first division still persists.

Smilacina racemosa

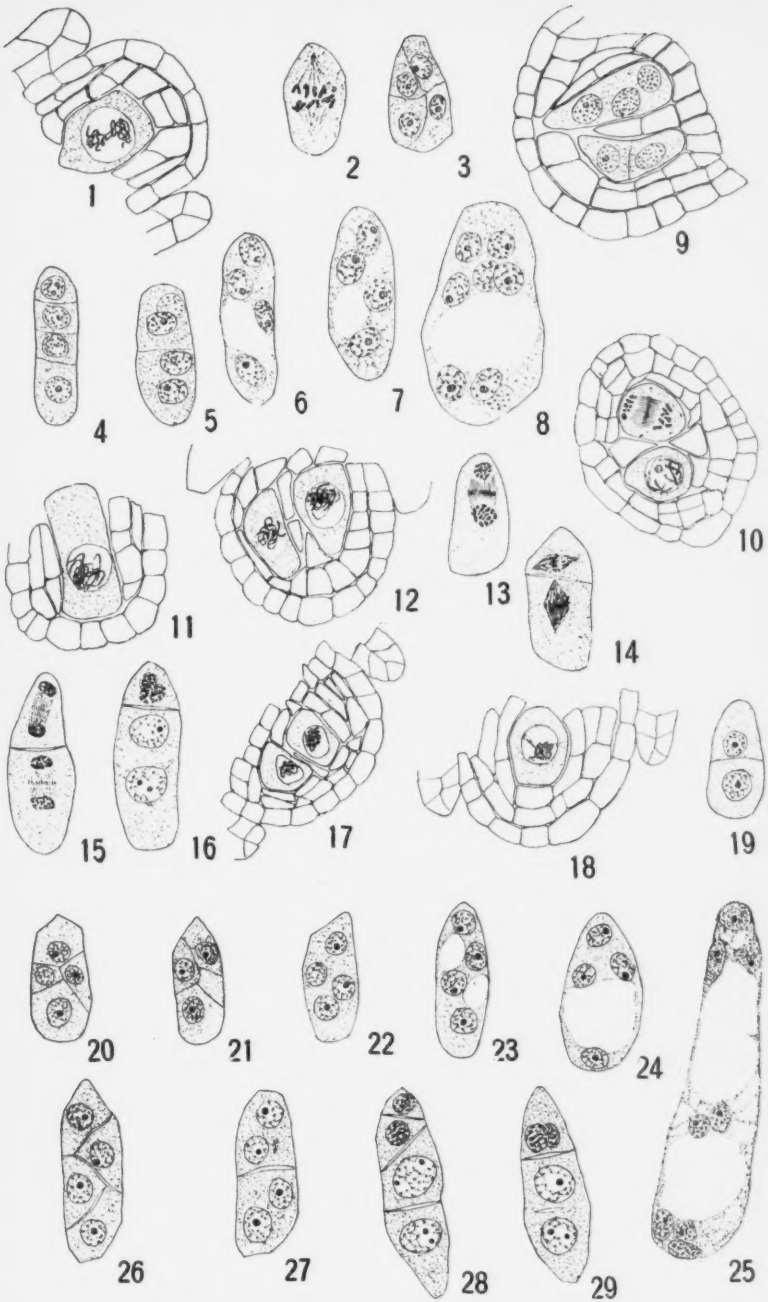
FIG. 28.—Four unequal megaspores fully separated by split cell plates.

FIG. 29.—A slightly later stage than the above, in which the cell membranes of the second division are disappearing; the inner pair of nuclei are no longer separated, while but a small part of the cell plate persists between the outer pair.

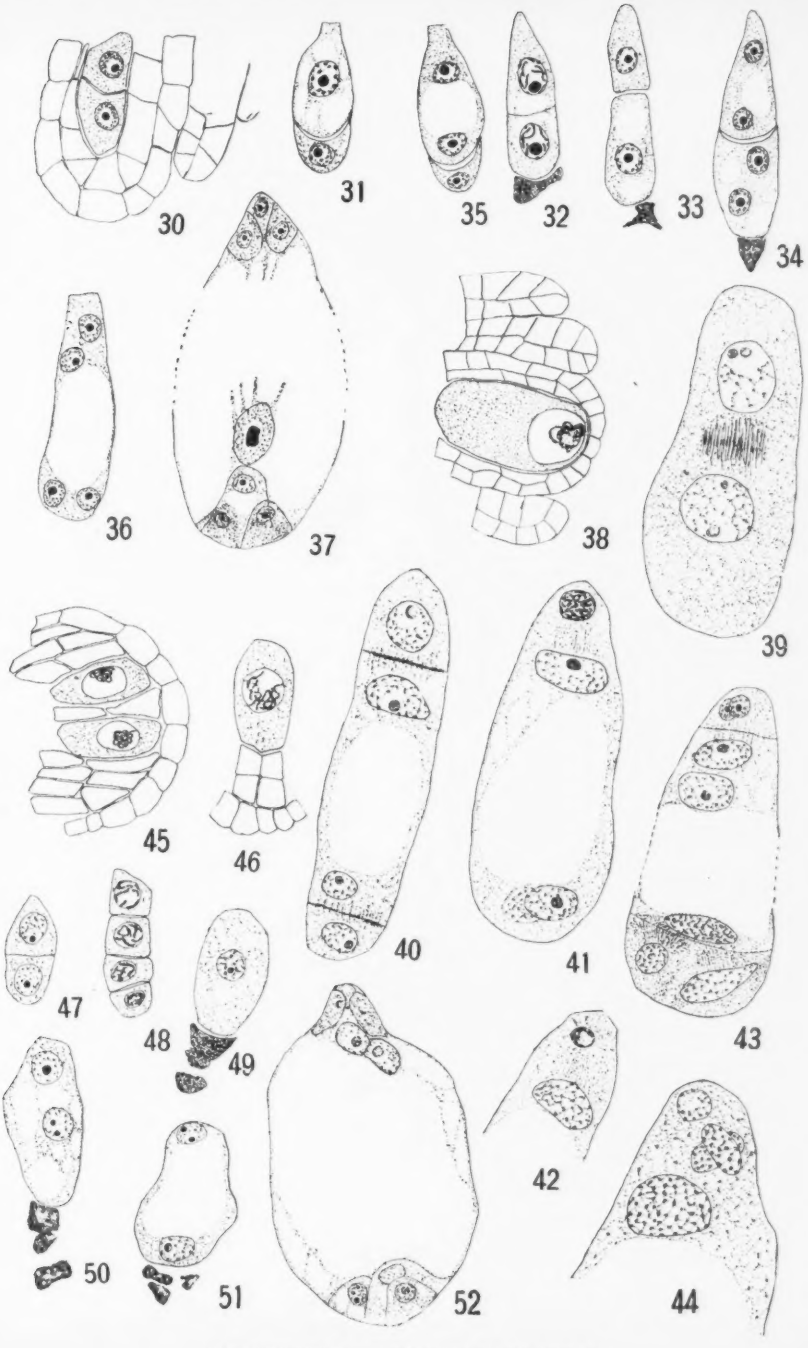
Streptopus roseus

FIG. 30.—Two fully separated daughter cells arising from the megaspore mother cell.

FIG. 31.—The inner daughter cell is enlarging at the expense of the outer.



McALLISTER on CONVALLARIACEAE



McALLISTER on CONVALLARIACEAE

FIG. 32.—The inner daughter cell has divided to form 2 cells which are separated by a definite and complete cell plate; the outer daughter cell now but a disorganized mass.

FIG. 33.—The 2 daughter cells of the second division widely separated by a split cell plate.

FIG. 34.—The nuclei of 2 fully separated inner daughter cells have divided, thus forming 2 binucleate cells; since both inner cells are to be regarded as megaspores, these 2 binucleate cells are immature female gametophytes.

FIG. 35.—A binucleate embryo sac with no trace of cell membranes.

FIG. 36.—A 4-nucleate stage of the embryo sac.

FIG. 37.—A fully formed embryo sac.

Medeola virginica

FIG. 38.—A megasporangium showing a single hypodermal megaspore mother cell, in the synaptic stage; magnification but half that of the other figures.

FIG. 39.—The first division of the mother cell; a conspicuous though incomplete cell plate is present.

FIG. 40.—The second division of the mother cell; the cell plate of the first division no longer visible; those of the second division very conspicuous but not sharply defined; the chalazal nuclei are practically equal.

FIG. 41.—A slightly earlier phase in which the inner pair of nuclei has a small inner (chalazal) nucleus and a large outer nucleus.

FIG. 42.—An embryo sac in which the inner pair of nuclei is very unequal; the inner nucleus stains deeply as though degenerating.

FIG. 43.—The third division; 2 small nuclei and 2 large ones in the antipodal end of the sac as a result of the unequal division of the antipodal nucleus in the previous division; the cell membranes of the second division still visible.

FIG. 44.—Antipodal region of the embryo sac showing 3 equal antipodal cells and the single fusion nucleus, fusion of the polar nuclei having taken place.

Polygonatum commutatum

FIG. 45.—Two hypodermal mother cells in a single nucellus.

FIG. 46.—A single mother cell showing two layers of cells between it and the epidermis.

FIG. 47.—Two daughter cells of the first division of the mother cell.

FIG. 48.—An axial row of 4 megaspores.

FIG. 49.—The enlarged inner megaspore and the disintegrated remnants of the outer three.

FIG. 50.—First division of the megaspore.

FIG. 51.—Binucleate embryo sac with a single large central vacuole.

FIG. 52.—Mature embryo sac; the polar nuclei not yet fused.

COMPARATIVE MORPHOLOGY OF SOME LEGUMINOSAE

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 190

JOHN N. MARTIN

(WITH PLATES VIII–XI)

This investigation covers the development of the embryo sac, embryo, and endosperm of *Trifolium pratense*, *T. hybridum*, *T. repens*, *Medicago sativa*, and *Vicia americana*. The work was done at the University of Chicago and Iowa State College. I wish to express my thanks to Professor COULTER and Professor CHAMBERLAIN who supervised the work, and to Professor PAMMEL at Iowa State College for valuable suggestions.

Historical

In 1839 and 1842 SCHLEIDEN and VOGEL (**8**) described the floral development and the endosperm of the Leguminosae. They investigated the endosperm of more than 50 species well distributed through the group. They found only a little endosperm remaining in *Trifolium* and *Medicago*, and none in *Vicia*. In 1855 TULASNE (**13**) described the proembryo of *Lathyrus Aphaca;* it produced a long filamentous suspensor and a distinct terminal embryo. HOFMEISTER (**5**) in 1858 described the embryo sac and proembryo of some species of *Lotus*, *Tetragonolobus*, *Trifolium*, *Lupinus*, and *Lathyrus;* he found a short massive proembryo in *Trifolium*. In 1880 STRASBURGER (**10**) described a proembryo developing a long suspensor in several species of *Lupinus* and in *Orobus vernus;* the cells of the suspensor are multinucleate and often separate. In the same year HEGELMAIER (**4**) reported the results of his investigations of the embryo sac, proembryo, and endosperm in four species of *Lupinus*. He found that the cells of the filamentous suspensor are multinucleate and that ephemeral cell plates appear during the division of the endosperm nuclei. In 1881 GUIGNARD'S (**3**) work appeared, covering about 40 species of the Leguminosae,

well distributed through the family. At that time the homology between microsporangium and megasporangium had not been established, and the terms "apical" and "subapical" were used to designate the primary parietal and sporogenous cells. His account shows that a one-celled archesporium prevails through the group. The archesporial cell always cuts off one parietal cell, which may remain undivided or produce a tissue varying in amount. The megaspore mother cell may produce the embryo sac directly, or form an axial row of 2, 3, or 4 cells, of which the innermost or one next to it may function. The embryo sac is always 8-celled and the antipodals are ephemeral. The fusion of the polar nuclei may occur in the center of the sac, against the inner wall, or in contact with the egg apparatus. The first division of the egg is transverse. The following divisions may result in a massive proembryo with no distinct line between the massive suspensor and embryo, or in a proembryo with a slender suspensor and a sharply defined terminal embryo. In 1907 SAXTON (7) described the embryo sac of *Cassia tomentosa*, and found one *deeply* buried megaspore mother cell. The third megaspore out of the longitudinal row of 4 functions; the polars fuse early; and the antipodals are persistent and form an absorptive tissue which fills the tubular projection in the chalazal region.

In 1912 COMPTON (1) described the seedling structure of 201 species of Leguminosae, ranging through all the regions of the vast family. He found the seedling epigeal in *Trifolium* and *Medicago*, but hypogeal in *Vicia*.

Material

Several fixing agents were tried, but chromo-acetic acid gave best results; one-half of 1 per cent strength was most satisfactory. The heads of *Trifolium* were split and entire halves run through and sectioned. Since the head is a compact raceme, this method enables one to trace the development of the embryo sac more readily, and proved to be of valuable assistance in determining the sterility of ovules. Separate flowers of *Medicago* and *Vicia* were used. The sections were stained with safranin and gentian violet.

Trifolium pratense

A more thorough study was made of this species because of its economic importance, and it was hoped that a better knowledge of its morphology would aid in controlling seed production. The discussion of *Trifolium pratense* will be somewhat detailed, and will form a basis for a comparative treatment of the other 4 species.

The ovary develops invariably 2 campylotropous ovules which are attached to a cushion-like placenta (fig. 1). The outer integument, which is barely a distinct ridge when the inner appears, soon passes the inner and forms a heavy rim about the micropyle (figs. 8, 10). The outer integument, which varies from 2 to 4 rows of cells in thickness at fertilization, except at the micropyle, thickens by periclinal divisions to many rows after fertilization, and the outer row of cells forms the peculiarly thickened and cutinized layer of the testa. The inner integument usually remains two layers in thickness until destroyed by the embryo sac. Sometimes three layers are seen in its basal region (fig. 10).

The nucellus is hemispherical at the time the archesporium can be identified, but elongates rapidly and is quite slender at the time the embryo sac is mature (fig. 12). There are usually 3 subepidermal rows (fig. 3), but often 3–6 rows are found at the base of the nucellus (fig. 12).

EMBRYO SAC.—From 1 to 4 archesporial cells were observed, and more than half of the ovules showed more than one archesporial cell. In fig. 3 there are 4 archesporial cells in a longitudinal row. In fig. 4 there are 4, but 3 are hypodermal and the other is more deeply placed. In either case, it is probable that the 4 archesporial cells have come from the division of a hypodermal cell at an early stage in the development of the nucellus. It is also probable that the cells deeper than the hypodermal layer may become sporogenous. One parietal cell is cut off (fig. 2), which usually divides transversely to form a longitudinal row of 2 or 3 cells (fig. 8), or one transverse and one longitudinal division may occur (fig. 6). No division (fig. 7) or only a longitudinal division has been observed. In fig. 5 two of the three megaspore mother cells have reached the synapsis stage, but more than one row of megaspores was not found.

So far as observed, a longitudinal row of 4 megaspores is always formed and the lower one functions (figs. 7, 8).

No chromosome counts were made, but the number is small and probably the same as in *Trifolium repens*, where it is about 12. The functioning megaspore rapidly destroys the surrounding tissue. The encroachment is at first most rapid at the chalazal end and results in a tubular sac (figs. 9, 10). As the megaspore enlarges, the tip of the nucellus thickens and with the surrounding portion of the inner integument becomes packed with starch (fig. 10). The megaspore divides in the direction of the long axis of the sac and the daughter nuclei pass to opposite poles (fig. 9). The following divisions may be parallel or transverse to the long axis of the sac (figs. 10–12). The longitudinal divisions are no doubt associated with the narrowness of the sac and occur most often in the antipodal end. As the embryo sac matures, it destroys the micropylar end of the nucellus and becomes much larger in this region (fig. 11). When the sac is mature, only the basal portion of the nucellus remains and the embryo sac lies against the inner integument (fig. 13). Sometimes the nucellus is more persistent, as shown in fig. 12.

The polar nuclei usually meet on the median line of the sac close to the egg apparatus (fig. 12), but occasionally they lie against the inner wall of the sac. Fusion awaits fertilization, but was found to occur if fertilization was prevented. Fig. 14 shows the fusion of the two polars before fertilization. In this case pollination was prevented and the ovaries were killed soon after the flowers began wilting.

The synergids often show a distinct filiform apparatus (fig. 14), which becomes more prominent if fertilization is prevented. The antipodals are ephemeral and no trace of them is left at the time of fertilization.

FERTILIZATION.—The fusion of the sex nuclei was not seen, although many flowers were pollinated and killed at various periods after pollination. The time between pollination and fertilization varies. Flowers pollinated during the high temperature of July and killed 18 hours after pollination showed pollen tubes entering, egg in first division, and 3-celled embryos. In October, when the

temperature was much lower, the time of fertilization ranged from 35 to 50 hours. This difference, no doubt, is partly due to a difference in growth conditions, and partly to delayed germination of the pollen. During the cooler weather pollen placed on the stigma at 3:00 P.M. was found dormant at 9:00 A.M. on the following day. This delayed germination will cause a marked difference in the time of fertilization. The pollen tube enters around the wall or in the region of a synergid (fig. 15); its behavior with reference to the synergid was not determined.

EMBRYO.—The first divisions of the egg are transverse (fig. 17), and result in a filament of three cells (fig. 18). By vertical walls in two planes this filament is divided into tiers of 4 cells each (fig. 19). The basal tier and a part of the second tier remain less active, but later form a massive suspensor (figs. 20, 21). No distinct line between embryo and suspensor was made out. In fig. 20 the dermatogen is being differentiated, which occurs later than the octant stage.

ENDOSPERM.—The division of the endosperm nucleus usually precedes that of the egg (fig. 15), but occasionally follows it (fig. 16). In fig. 17 the fertilized egg has completed its first division, and 5 endosperm nuclei were counted. The endosperm masses about the embryo and from this mass it extends around the wall of the sac. Its later development is centripetal. Only the first division of the endosperm nucleus was seen in the many ovules studied. This fact indicates that the divisions in the endosperm nuclei are simultaneous, but this feature was not determined.

STERILITY.—The sterility of ovules is a prominent feature in *Trifolium pratense*. In the sterile ovules all the cells of the nucellus remain vegetative and hence no embryo sacs are found. All the flowers of a plant frequently develop sterile ovules only. This seems to be related to moisture conditions, but more work is necessary before a definite conclusion can be drawn as to its cause. Plants grown in the greenhouse and well watered gave 100 per cent sterile ovules. First crop heads collected from the field during wet weather showed nearly 100 per cent sterility, while first crop heads collected after two weeks of dry weather showed a large percentage of fertile ovules. But even during dry weather

there is considerable variation among plants, some producing nearly all fertile ovules, and others a large percentage of sterile ones. There is a marked tendency toward sterility, which seem to be favored by moisture. This tendency, no doubt, always lowers the percentage of seed production, and in some cases reduces it almost to zero. The fact that this tendency varies among plants under similar conditions suggests that it may be partly eliminated by selection. A sterile ovule is shown in fig. 22. The flower was open and the embryo sac should have been ready for fertilization. Sterile ovules can be identified only in later stages. The cells of the subepidermal rows are usually larger and less dense in content, but it is safe to pass judgment on the earlier stages only when all the later stages of the head are sterile. No mother cells in synapsis were found in the sterile ovules, so sterility seems to be determined before this stage is reached.

PARTHENOGENESIS.—*Trifolium pratense* has been reported parthenogenetic. Flowers were run under cover and killed at various times after wilting. An examination of more than 500 ovaries showed no embryos. The ovule enlarges very rapidly for several days after the embryo sac is ready for fertilization and then begins to break down.

Trifolium hybridum

This species is so similar to *Trifolium pratense* that a few features only deserve mention. The number of ovules in an ovary is variable, ranging from 3 to 8. Fig. 23 shows the lower megaspore germinating before the others are destroyed. The embryo sac has a large central vacuole and the cytoplasm is almost entirely limited to a thin peripheral layer (fig. 24). The embryo sac becomes more curved than that of *Trifolium pratense* (fig. 27). The polars fuse in a parietal position (fig. 24). The proembryo is more slender and there is a more definite line between embryo and suspensor in the later stages (fig. 25). Fig. 25 also shows the faint walls that sometimes occur in the early development of the endosperm. Fig. 26 shows the suspensor on the hypocotyl of the embryo. The tendency toward sterility is not so pronounced in this species as it is in *Trifolium pratense*.

Trifolium repens

This species agrees very closely with *Trifolium hybridum*. Fig. 28 shows the reduction division of the mother cell; 11 chromosomes are shown, but various counts gave 11 and 12. The number is small, about 12. In fig. 29 one megaspore mother cell has produced 4 megaspores, while the other one has enlarged but has made no division. The third megaspore often functions (fig. 30). The embryo does not differ from that of *Trifolium hybridum*. The endosperm masses about the embryo in its early development, but no walls occur. Not much sterilization was observed.

Medicago sativa

In *Medicago sativa* the number of ovules in an ovary varies considerably, ranging between 12 and 18. The nucellus is more massive than that of *Trifolium*. The number of subepidermal rows ranges from 5 to 7 (figs. 31, 34). The outer integument precedes the inner as in *Trifolium*. The number of archesporial cells ranges from 1 to 6, and more than one usually occurs (fig. 31). One parietal cell is nearly always cut off, but occasionally megaspore mother cells may be found with no parietal cells (fig. 32). The parietal cell usually makes only one division, which is transverse (figs. 32, 33), but at times the transverse division is followed by one or more longitudinal ones (fig. 34). Prominent cell plates accompany the formation of the megaspores (figs. 33, 34). From 2 to 4 rows of megaspores may occur in the same nucellus (fig. 34) and often more than one megaspore starts to form an embryo sac (fig. 35), but not more than one mature sac was found. The embryo sac destroys the surrounding nucellar tissue more uniformly and does not become so tubular as in *Trifolium* (figs. 36, 37). The large central vacuole which appears during the formation of the 8 nuclei (fig. 36) disappears later, and the cytoplasm becomes compact and filled with starch (fig. 37). The 8 nuclei of the embryo sac are arranged in two groups of 4 each, separated by the large vacuole (fig. 36), and when the embryo sac is mature, 3 nuclei of each group are definitely set off at the poles in separate masses of cytoplasm, while the 2 polar nuclei occupy the median mass of cytoplasm (fig. 37). The polars meet near the middle of the sac

(fig. 37) and then move to a position near the egg apparatus (fig. 38), where they fuse late, probably not until fertilization. Three antipodals are usually formed (fig. 36), but sometimes only one division occurs in the antipodal end, which results in only one antipodal cell (fig. 37). The antipodals disappear before the embryo sac is mature (fig. 38).

EMBRYO.—The egg forms a filament of 5 cells, the terminal one of which produces the embryo, while the other 4 constitute the suspensor (figs. 39, 40). The basal cell of the suspensor is quite long. All the cells have thin, granular cytoplasm and are usually multinucleate. The suspensor is still seen on the advanced embryo (fig. 41). Fig. 40 shows the embryo with dermatogen already differentiated.

ENDOSPERM.—The endosperm takes the parietal arrangement with the earlier development in the micropylar end, but does not mass about the embryo as in the clovers (fig. 40). No sterility was observed in *Medicago sativa*, but all material used, so far, was collected during rather dry periods, and further investigation is necessary to determine the effect of moisture upon its fertility.

Vicia americana

The number of ovules in *Vicia americana* has about the same range as in *Medicago sativa*. The nucellus is smaller, usually having 3 or 4 subepidermal rows. Periclinal divisions are rapid in the early stage of the nucellus, which give it a slender form. The outer integument, as in *Trifolium* and *Medicago*, precedes the inner. Fig. 42 shows 5 archesporial cells, all of which are probably not of hypodermal origin. The development of parietal tissue is variable, as shown by figs. 43–45. A row of 4 megaspores is formed (fig. 44) and the lower functions (fig. 45).

The embryo sac resembles that of *Medicago sativa* in formation, shape, and in the destruction of nucellar tissue. The antipodals are ephemeral as in the other species (figs. 46, 47), but the position of the polars is near the inner wall in the middle of the sac (fig. 47). It resembles the clover in having much starch in the inner integument and little in the sac.

EMBRYO.—The proembryo consists of a filament of three cells, of which the terminal forms the embryo, while the other two form a long suspensor of two tiers of two cells each. The cells of the suspensor have very little cytoplasm and are multinucleate (figs. 48–51).

ENDOSPERM.—The endosperm nucleus and egg divide at about the same time. The endosperm nuclei divide rapidly and simultaneously. The endosperm is parietally placed and does not mass about the proembryo in the early stages of its development (figs. 52, 53). Some sterile ovules were found, but the tendency toward sterility is not so pronounced as in the clovers.

Discussion

GUIGNARD'S account of *Trifolium* (**3**, pp. 119, 120) is limited to the development of the embryo. The fertilized egg produces a filament of 3 cells, the terminal of which develops the embryo. The other 2 cells produce a short, several-celled suspensor. In the later stages the line of separation between embryo and suspensor is indistinct. The dermatogen is differentiated in the octant stage. In the three species of *Trifolium* investigated by the writer, the suspensor is more massive and the dermatogen is differentiated later than the octant stage.

In *Medicago arborea* GUIGNARD (**3**, pp. 119, 120, *figs. 192–194*) reports a one-celled archesporium and two superimposed parietal cells. The megaspore mother cell functions directly to produce the embryo sac. The proembryo produces a long, filamentous suspensor which is distinct from the terminal embryo. The dermatogen is differentiated in the octant stage. In *Medicago sativa* there is usually a multicellular archesporium, production of megaspores, and more parietal tissue, but in other features it is similar to *Medicago arborea*. In *Vicia sepium*, GUIGNARD (**3**, p. 53, *figs. 66–70*) found one parietal cell which occasionally makes one transverse division. The single megaspore mother cell produces a longitudinal row of 3 cells, the lower 2 being megaspores, or a longitudinal row of 4 megaspores; in each case the lowest megaspore functions. The proembryo produces a long suspensor which consists of two rows of cells and is distinct from the embryo. In *Vicia americana* a

multicellular archesporium usually occurs, and so far as observed a longitudinal row of 4 megaspores is always formed.

GUIGNARD gives no account of a multicellular archesporium in any of the species which he studied, but the early stages of the ovules were examined without sectioning, and it is probable that present methods would give different results. He found parietal tissue in all species studied, but the greatest amount in the Mimosoideae and Caesalpinioideae.

The following records show that a multicellular archesporium occurs in other families of the Rosales. Miss PACE (**6**) found a multicellular archesporium in *Parnassia* and *Saxifraga*. WEBB (**14**) reported the same type in *Astilbe*. SHOEMAKER (**9**) found several archesporial cells in *Hamamelis;* and COULTER and CHAMBERLAIN (**2**, pp. 58, 59) in a summary of the literature show that a multicellular archesporium prevails among the Rosaceae.

The filiform apparatus in *Trifolium pratense* differs from that described by PACE in *Parnassia* and *Saxifraga* and by STRASBURGER in *Polygonum* (**11**) and *Santalum* (**12**), in that no notch appears.

GUIGNARD (**3**, p. 142) states that the polar nuclei fuse before fertilization except in the subfamily Vicieae, and that the fusion nucleus rests on the median line of the sac in the Mimosoideae and Caesalpinioideae, and against the inner side of the sac in the Papilionoideae. In the five species treated in this paper, the fusion of the polar nuclei was found to await fertilization, and their position is median in *Medicago sativa*, but may be either median or parietal in the species of *Trifolium*, and always parietal in *Vicia americana*.

GUIGNARD (**3**, p. 141) found the antipodal cells persisting till fertilization in the Mimosoideae and Caesalpinioideae, but disappearing earlier in the Papilionoideae. SAXTON (**7**) found the antipodals persistent and functioning as haustoria in *Cassia tomentosa*.

Summary

Features common to the five species are as follows: (1) campylotropous ovules; (2) two integuments, the outer preceding the inner; (3) a multicellular archesporium; (4) one parietal cell cut off which gives rise to more or less parietal tissue; (5) the production of a row of 4 megaspores; (6) the rapid destruction of

nucellar tissue which brings the embryo sac in contact with the inner integument; (7) antipodals ephemeral.

Contrasting features are as follows: (1) the number of ovules is always 2 in *Trifolium pratense*, but more than 2 and various in the other species; (2) the third megaspore sometimes functions in *T. repens;* (3) in *Trifolium* the embryo sac rapidly destroys the antipodal end of the nucellus and thus forms a long tubular sac; (4) in *Trifolium* the embryo sac remains very vacuolate, while in *Vicia* and *Medicago* the sac fills with cytoplasm; (5) polars meet on median line or on inner side of sac in *Trifolium*, but rest near the egg apparatus; in *Medicago* the polars meet near the center of the sac and rest near the egg apparatus; while in *Vicia* they meet on the inner side of the sac and remain some distance from the egg apparatus; (6) in *Trifolium* and *Vicia*, the starch appears in the micropylar end of the nucellus and in the inner integument, while the starch fills the sac in *Medicago;* (7) in *Trifolium* the proembryo is short and massive and no definite line between suspensor and embryo was made out; more evidence of the separate parts was seen in the more slender proembryos of *T. hybridum* and *T. repens;* (8) definite suspensors with multinucleate cells appear in *Medicago sativa* and *Vicia americana;* in the former species the suspensor is filamentous, but composed of two superimposed pairs of cells in the latter species; (9) sterilization is most marked in *T. pratense*.

IOWA STATE COLLEGE
AMES, IOWA

LITERATURE CITED

1. COMPTON, R. H., An investigation of the seedling structure in the Leguminosae. Jour. Linn. Soc. Bot. **41**:1–122. *pls. 9.* 1912.
2. COULTER, JOHN M., and CHAMBERLAIN, CHARLES J., Morphology of angiosperms. New York. 1903.
3. GUIGNARD, L., Recherches d'embryogénie végétale comparée. I. Legumineuses. Ann. Sci. Nat. Bot. VI. **12**:5–166. *figs. 241.* 1881.
4. HEGELMAIER, F., Zur Embryogenie und Endospermentwickelung von *Lupinus*. Bot. Zeit. nos. 5–9. *pls. 2.* 1880.
5. HOFMEISTER, WILHELM, Embryobildung der Phanerogamen. Jahrb. Wiss. Bot. **1**:101–103. *figs. 1–41.* 1858.
6. PACE, LULA, *Parnassia* and some allied genera. BOT. GAZ. **54**:307–329. *pls. 14–17.* 1912.

7. SAXTON, W. T., On the development of the ovule and embryo sac in *Cassia tomentosa*. Trans. S. Afr. Phil. Soc. **18**:1–5. *pls. 1, 2*. 1907.
8. SCHLEIDEN, M. J., and VOGEL, T., Über das Albumen inbesondere der Leguminosen. Kaiserl. Leopold.-Carol. Deutsch. Akad. **19**: pts. 1 and 2. 1838 and 1842.
9. SHOEMAKER, D. N., Notes on the development of *Hamamelis virginiana*. Johns Hopkins Univ. Circ. **21**:86, 87. 1902.
10. STRASBURGER, E., Einige Bemerkungen über vielkernige Zellen und Embryogenie von *Lupinus*. Bot. Zeit. **38**:845–854, 857–868. *figs. 64*. 1880.
11. ———, Die Angiospermen und die Gymnospermen. Jena. 1870.
12. ———, Zur *Santalum* und *Daphne*. Ber. Deutsch. Bot. Gesells. **3**:105–113. *pl. 9*. 1885.
13. TULASNE, M., Nouvelles études d'embryologie végétal. Ann. Sci. Nat. Bot. IV. **4**:96, 97. 1855.
14. WEBB, J. E., A morphological study of the flower and embryo of *Spiraea*. BOT. GAZ. **33**:451–460. *figs. 28*. 1902; and BOT. GAZ. **34**:246. 1902.

EXPLANATION OF PLATES VIII–XI

Trifolium pratense

FIG. 1.—Young ovary showing the two ovules and placenta; ×330.

FIG. 2.—Young nucellus showing one sporogenous and one parietal cell; ×1560.

FIG. 3.—Young nucellus with a row of 4 archesporial cells; both integuments are prominent; ×1500.

FIG. 4.—Young nucellus with 4 archesporial cells; inner integument is just beginning to show; ×1560.

FIG. 5.—Cross-section of nucellus with 3 megaspores, 2 of which are in synapsis; ×1500.

FIG. 6.—Young nucellus with one mother cell in synapsis; the integuments are well advanced; ×1200.

FIG. 7.—Nucellus with a row of 4 megaspores; two large dense cells lie in line below the megaspores; parietal cell has not divided; ×1200.

FIG. 8.—Four megaspores with lower one functioning; ×1500.

FIG. 9.—A binucleate embryo sac eating its way into the chalazal end of the nucellus; ×1560.

FIG. 10.—A 4-nucleate embryo sac; the divisions at each end have been in the direction of the long axis of the sac; integuments well advanced and outer is thickened at micropylar end; the ends of the nucellus and the inner integument are filled with starch; ×880.

FIG. 11.—An 8-nucleate sac, with linear arrangement at antipodal end; micropylar end of nucellus being destroyed; embryo sac quite vacuolate; ×920.

FIG. 12.—Polars in contact near the egg apparatus; nucellus more persistent; ×1200.

FIG. 13.—Embryo sac mature and in contact with inner integument; ×920.

FIG. 14.—Egg apparatus with synergids showing filiform apparatus; the nucleoli of the polars are fusing; ×1560.

FIG. 15.—Pollen tube in region of synergid, and egg in division; endosperm cell has made one division; ×920.

FIG. 16.—A 2-celled proembryo, while the endosperm cell is in first division; ×920.

FIG. 17.—A 2-celled proembryo; 5 endosperm nuclei were counted; ×920.

FIG. 18.—A 3-celled proembryo; ×920.

FIG. 19.—Proembryo has divided by vertical walls; ×750.

FIG. 20.—Dermatogen is cut off; ×750.

FIG. 21.—The massive proembryo with no line of separation between embryo and suspensor; ×540.

FIG. 22.—Median longitudinal section of a sterile ovule; embryo sac replaced by vegetative cells; ×1200.

Trifolium hybridum

FIG. 23.—Early germination of lower megaspore; ×1500.

FIG. 24.—An 8-nucleate embryo sac; cytoplasm of the sac forms a marginal layer around the large vacuole; polars in contact on inner side of sac; ×910.

FIG. 25.—Proembryo with cotyledons appearing; the stalk is composed of large cells, and there is an apparent line of separation between suspensor and embryo at this stage; ×540.

FIG. 26.—Remains of suspensor on the hypocotyl of the embryo; ×530.

FIG. 27.—Ovule showing the long, curved embryo sac, and proembryo in early stage; ×360.

Trifolium repens

FIG. 28.—Reduction division of mother cell; outer integument well developed; ×1500.

FIG. 29.—One row of megaspores and one enlarged mother cell; ×1500.

FIG. 30.—Third megaspore functioning; ×1200.

Medicago sativa

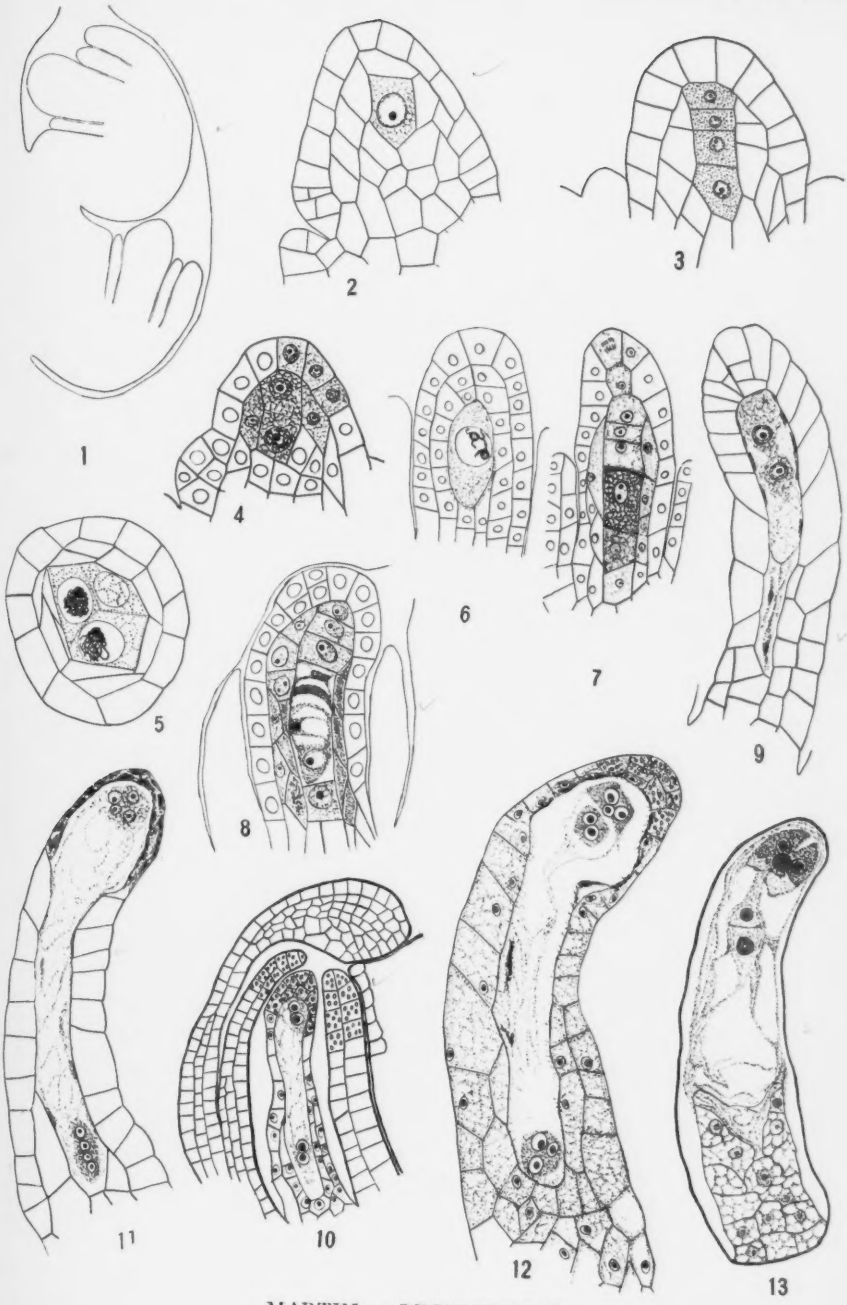
FIG. 31.—Young nucellus with 3 sporogenous cells, each capped by one parietal cell; ×1500.

FIG. 32.—Three mother cells in synapsis; two have no parietal cells; ×1500.

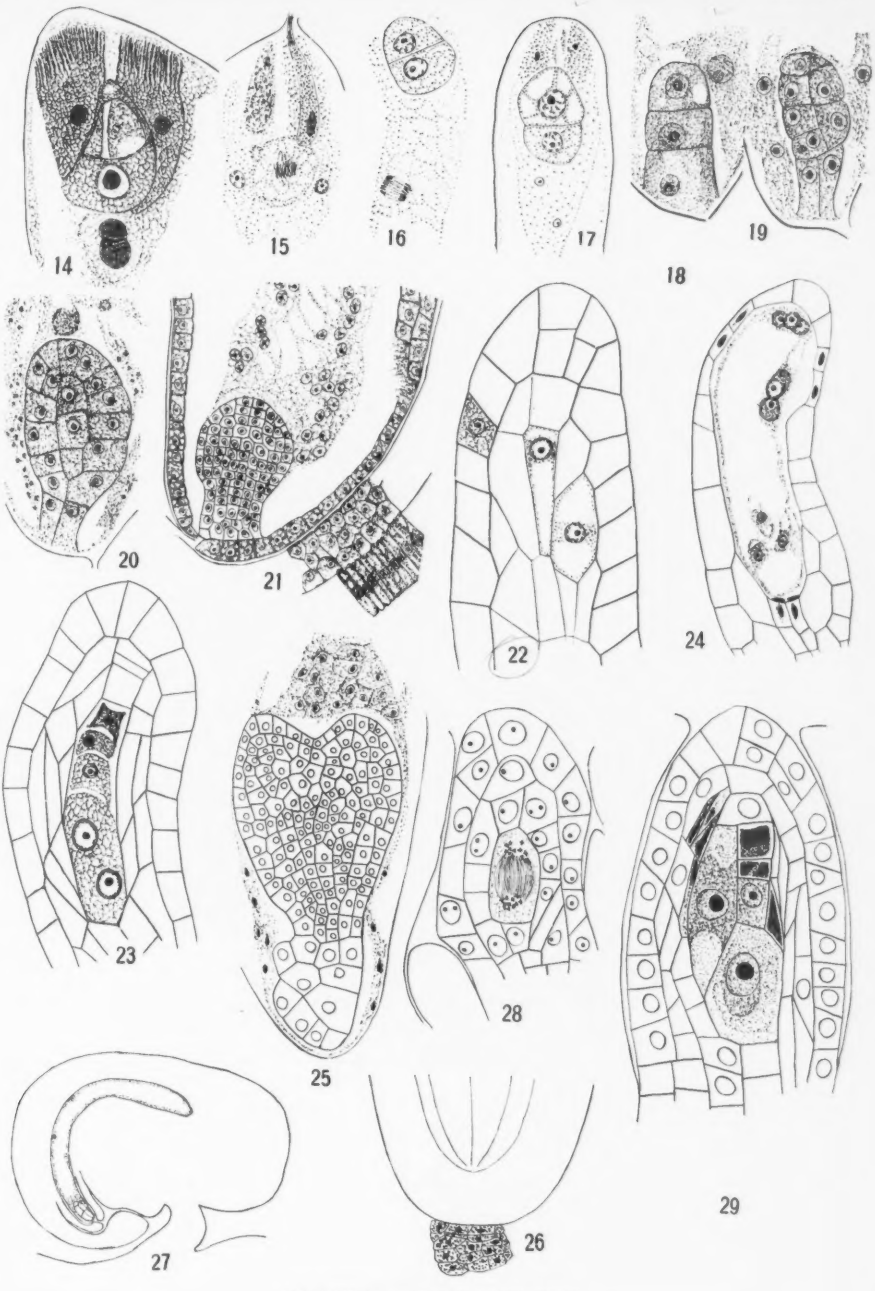
FIG. 33.—Mother cell in reduction division; parietal cell has divided transversely; ×1500.

FIG. 34.—Two rows of megaspores; ×1500.

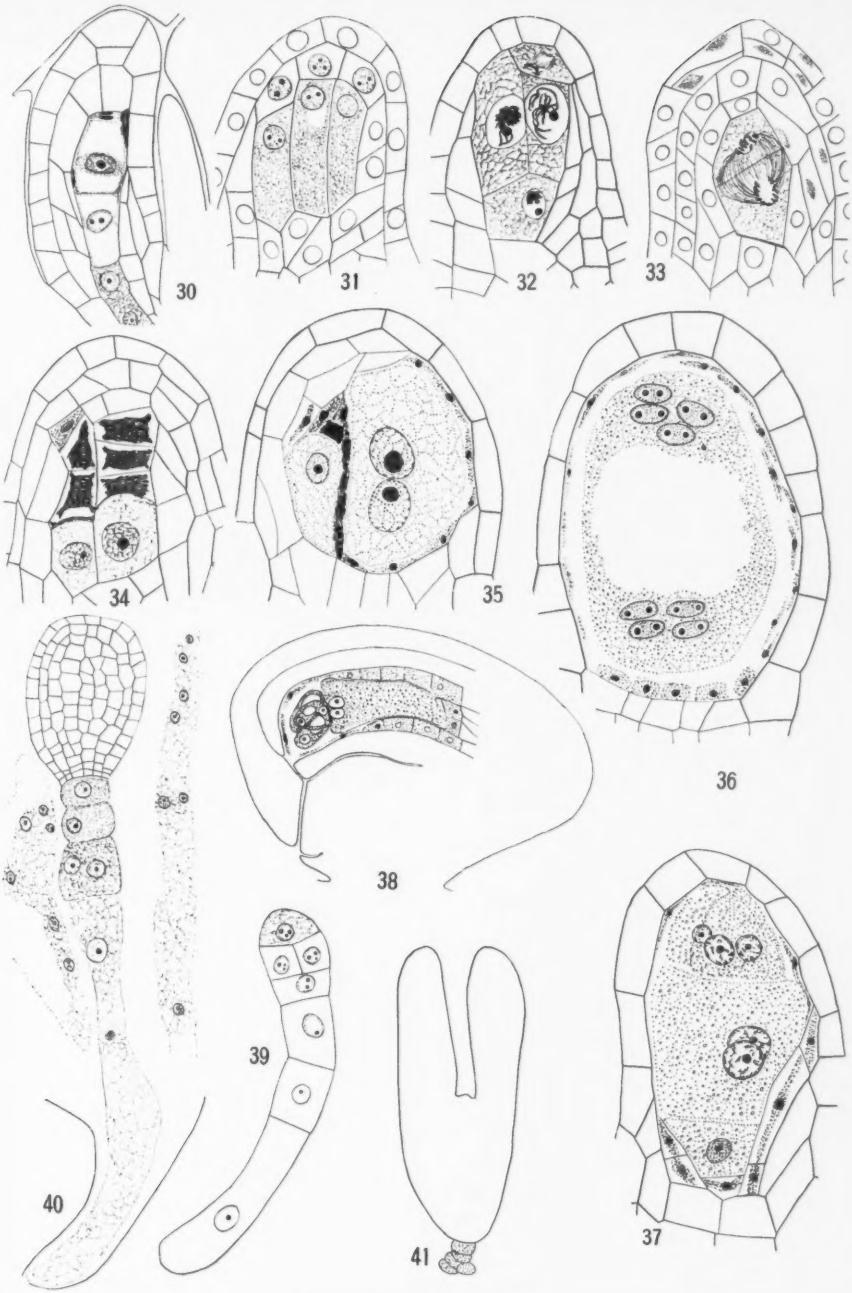
FIG. 35.—Two embryo sacs; one is binucleate; ×1500.



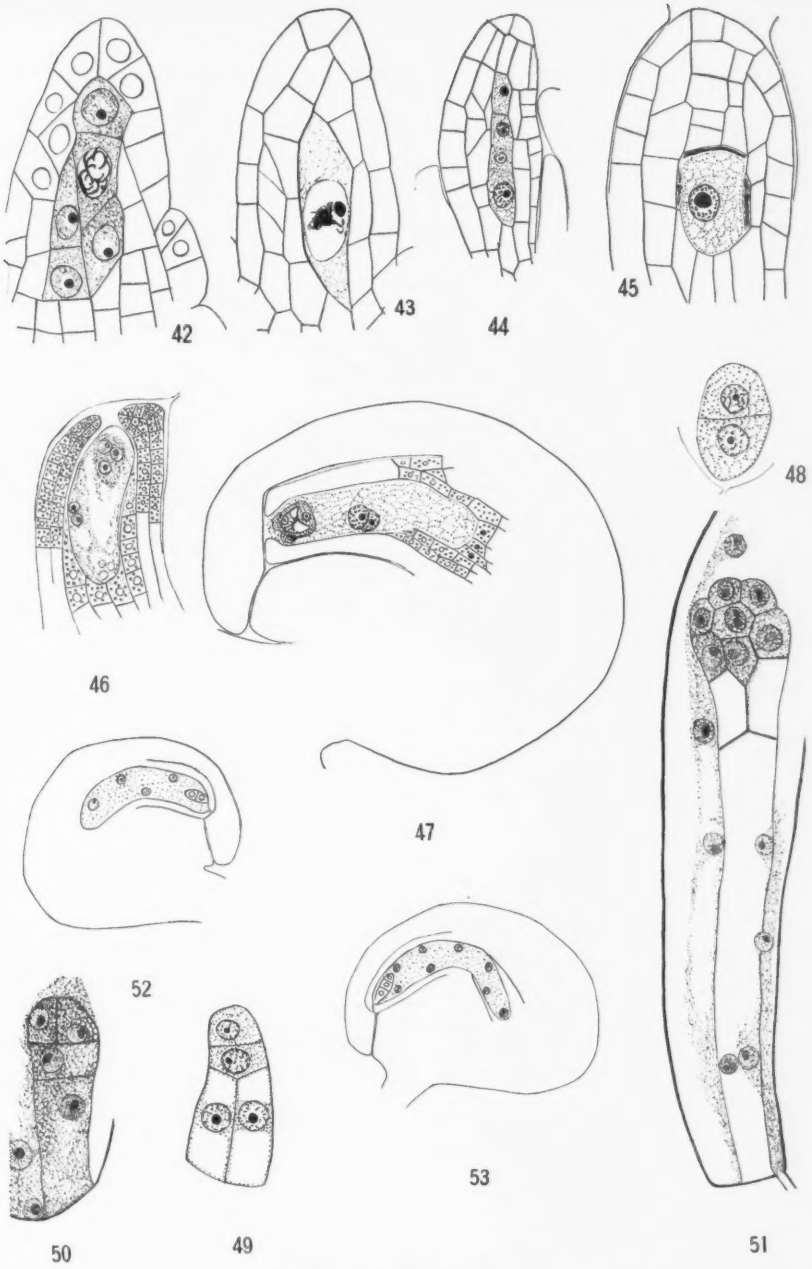
MARTIN on LEGUMINOSAE



MARTIN on LEGUMINOSAE



MARTIN on LEGUMINOSAE



MARTIN on LEGUMINOSAE

FIG. 36.—Embryo sac 8-nucleate, with large central vacuole and much starch; ×1500.

FIG. 37.—Vacuole in embryo sac has disappeared; polars in center of sac; sac is divided into three separate portions; only one antipodal cell; ×1500.

FIG. 38.—Mature embryo sac; polars in contact with egg apparatus; nucellus has disappeared at the micropylar end; ×360.

FIG. 39.—Proembryo with 4-celled suspensor; ×910.

FIG. 40.—Advanced stage of embryo; embryo has cut off epidermis; suspensor has thin cytoplasm and its cells are multinucleate; ×510.

FIG. 41.—Advanced embryo with suspensor showing on hypocotyl; ×360.

Vicia americana

FIG. 42.—Young nucellus with 5 archesporial cells; the integuments are appearing; ×1500.

FIG. 43.—Mother cell in synapsis; parietal cell has divided longitudinally; ×1200.

FIG. 44.—A row of 4 megaspores; ×750.

FIG. 45.—Functioning megaspore; much parietal tissue has developed; ×1500.

FIG. 46.—Mature embryo sac with nucellus eaten away; starch abundant in the inner integument and remaining portion of nucellus; polars on inner side of embryo sac and distant from egg apparatus; ×540.

FIG. 47.—The campylotropous ovule with mature embryo sac; ×330.

FIG. 48.—A 2-celled proembryo; ×1500.

FIG. 49.—A 4-celled proembryo; apical cell produces the embryo; ×900.

FIG. 50.—Proembryo in a view showing only one cell of each pair of cells composing the suspensor and the divided apical cell which produces the embryo; ×1200.

FIG. 51.—Suspensor distinct from embryo; basal cells of suspensor much elongated and multinucleate; the cells of the suspensor have very little cytoplasm; endosperm is parietally arranged; ×1200.

FIG. 52.—A 2-celled proembryo and 4 endosperm nuclei; ×360.

FIG. 53.—A 3-celled proembryo and 8 endosperm nuclei parietally placed; ×360.

CONTRIBUTIONS TO THE ANATOMY OF MESOZOIC CONIFERS

NO. 2. CRETACEOUS LIGNITES FROM CLIFFWOOD, NEW JERSEY[1]

RUTH HOLDEN

(WITH PLATES XII–XV)

Araucarioxylon

One of the most interesting and, at the same time, most disputed questions in the phylogeny of the Coniferales deals with the relative antiquity of the abietineous and araucarian lines. The general ligneous structure of the latter has led the majority of botanists to its direct affiliation with the Cordaitales. In a recent paper, Professor JEFFREY[2] has shown that this resemblance exists only in the normal mature wood, and that in the seedling stem, root, cone axis, and traumatic tissue, there are present altogether different conditions. In these regions the rays often become thick-walled and heavily pitted, there are wood parenchyma cells, and the tracheary pits, instead of being closely approximated, are scattered and opposite. Moreover, in the cone axis the pits are separated by well marked cellulose bars of Sanio. All these features are characteristic of the Abietineae, and diametrically opposed to anything found in the Cordaitales. The locality of these digressions from the *Araucarioxylon* type is of especial significance. On both the zoological and botanical sides, the law of recapitulation is regarded as one of the fundamental conceptions of evolution, and according to this law it is in the plant seedling that ancestral features should be found. Further, case after case has been recorded where these primitive conditions are retained in the root and reproductive axis, and recalled in traumatic tissue. As

[1] Contributions from the Phanerogamic Laboratories of Harvard University, no. 63.

[2] JEFFREY, E. C., The history, comparative anatomy, and evolution of the *Araucarioxylon* type. Proc. Amer. Acad. **48**:531–571. *pls.* 7. 1912.

instances of the first may be mentioned the occurrence of resin canals in the center of the root of such conifers as *Abies* and *Tsuga*, a harking back to the time when they were scattered throughout the wood, as in *Pinus;* as instances of the second, the presence of centripetal wood in the cone axis of *Equisetum;* and of the last, the ray tracheids in wounded specimens of *Abies*. It seems clear, therefore, from comparative anatomical and developmental evidence, that the Araucarineae are descended from ancestors which had thick-walled pitted rays, wood parenchyma, and scattered tracheary pits separated by bars of Sanio. These hypothetical ancestors are probably the Abietineae.

If such a conclusion is correct, we should expect to find fossil record of woods which possessed these modified araucarian structures, not only in primitive places, but also in their normal, mature growth. Recent investigation has shown such to be the case. For example, *Araucarioxylon Lindleyi* Seward, *Cormaraucarioxylon crasseradiatum* Lignier, and *Araucarioxylon noveboracense* Jeffrey have wood parenchyma; *Cormaraucarioxylon crasseradiatum* Lignier, *Protocedroxylon araucarioides* Gothan, and *Araucariopitys americana* Jeffrey have thick-walled pitted rays. Further, many of these Mesozoic araucarians have traumatic resin canals, as do *Abies, Cedrus*, etc. Up to the present, however, no fossil araucarian has been described with opposite pits or bars of Sanio. In an earlier paper[3] the writer has described several species of *Pityoxylon* from the Raritan Cretaceous of Cliffwood, New Jersey. These were included in a considerable amount of lignite collected by Professor JEFFREY, and turned over to the writer for investigation. In addition to the *Pityoxyla*, there were a number of *Cupressinoxyla*, and one *Araucarioxylon*. This last specimen was of especial interest in this connection, and will accordingly be described first.

The material consisted of a flattened stem about two inches in length and one in diameter. The preservation of the outer layer was but indifferent; near the center, however, it was excellent. The pith contains large masses of stone cells, similar to those of the living *Agathis*. Fig. 1 represents a radial section of the wood at

[3] HOLDEN, RUTH, Cretaceous *Pityoxyla* from Cliffwood, New Jersey. Proc. Amer. Acad. **48**:609-623. *pls. 4.* 1913.

some distance from the pith; fig. 2, the same at a higher magnification. The general araucarian nature of the specimen is vouched for by the thin rays, absence of wood parenchyma, and especially by the alternating and closely compressed tracheary pitting.

The structure next the pith is elucidated by fig. 4. At the extreme left are the ringed protoxylem and metaxylem elements, which, toward the right, are replaced by those of the secondary wood. In the first pitted tracheid the pits are uniseriate and scattered; in the second they are opposite. A careful examination of the wall between the pairs of opposite pits shows that they are separated by white lines. Fig. 5 represents these same elements at a higher magnification, and here the lines are still more conspicuous. The study of fossil *Pityoxyla*, a section of which is shown in fig. 8, has proved that the bars of Sanio in those woods are represented by similar white lines, a condition which would be expected from the cellulose nature of the bars, and the tendency of cellulose structures to disappear in the process of fossilization. That the white lines appearing in figs. 4 and 5 are also bars of Sanio seems unquestionable. The general resemblance of the pitting of this Cretaceous stem to that of the cone axis of *Araucaria Bidwillii* (fig. 7) is very striking. In the case of the latter, the cellulose composing the bars is still present, and stains a dark blue with hematoxylon, causing the bars to stand out as black lines in the photograph. In studying unstained living material, it would be possible to mistake the spirals of the primary wood for bars of Sanio. This possibility may be obviated, as JEFFREY has demonstrated, by using a double stain of hematoxylon and saffranin. The former stains the bars a deep blue and leaves the spirals untouched, while the latter stains the spirals a bright red. With fossil material, however, there is no chance for mistake. Here the protoxylem spirals, being lignified, persist, and appear as black lines (left of fig. 4); on the other hand, the bars of Sanio, being cellulose, drop out, and appear as white lines (right of fig. 4).

Fig. 3 represents a similar section from the same Cretaceous specimen. Toward the extreme left may be seen indistinctly a tracheid with metaxylem spirals; next it is one with approximately opposite pits. Near the lower limit bars of Sanio may be made out.

In the next tracheid the pitting becomes uniseriate again, and the bars of Sanio stand out with considerable clearness. Still a third case is presented in fig. 6. At the left are two spiral elements, and next them is a pitted element. Toward the upper end of the latter the pits are biseriate, with faint bars of Sanio; below they become triseriate, and then biseriate again. In the latter case they are alternate, but even here bars of Sanio are present. A similar combination of alternating pitting and bars of Sanio is shown in the tracheid at the right of fig. 7 (cone of *Araucaria Bidwillii*), and similar triseriate pitting in that at the extreme left.

This type of pitting is uniformly present in this specimen near the pith. Accordingly it seems to supply the only missing link in the chain of evidence pointing to the derivation of the Araucarineae from the Abietineae. All the araucarian features of wood structure have been previously shown to disappear in the primitive regions of extant araucarians, and now they have all been shown to disappear in the stem of extinct ones.

Brachyoxylon

Of the remainder of the Cliffwood lignites, a considerable amount belongs to the genus *Brachyoxylon*. In some cases twigs were found similar in almost every respect to such specimens as *Geinitzia Reichenbachi*, *Brachyphyllum macrocarpum*, etc., from the Cretaceous of Kreischerville, New York.[4] Figs. 9 and 10 represent transverse and longitudinal sections of one of these. The tracheids have the same combination of araucarian and abietineous pitting, the rays are smooth-walled, there is no wood parenchyma, and, furthermore, there are abundant sclerites in the pith. The particular specimen figured here is so like *Geinitzia Reichenbachi* in the shape and arrangement of stone cells that it seems safe to identify it with that species.

In other twigs, however, the arrangement of these sclerenchymatous elements is quite different. In figs. 11 and 14, for example, they are grouped to form nests, extending like plates, sometimes almost completely across the medulla. As shown in the photo-

[4] HOLLICK, ARTHUR, and JEFFREY, E. C., Studies of cretaceous coniferous remains from Kreischerville, New York. Mem. N.Y. Bot. Garden 3:1–38. *pls. 1–29.* 1909.

graphs, the individual cells are elongated longitudinally, and filled with a dark substance. This stem was one of the few with the bark still adhering. Figs. 12 and 13 show the details of the bast, and it is evident that, although in other respects it resembles closely some of the Kreischerville material, in this respect it is quite different. In *Brachyphyllum*, *Araucariopitys*, etc., the phloem is composed entirely of sieve tubes and parenchyma cells, while here there are tangential rows of bast fibers. This alternation of hard and soft bast commonly obtains in the Cupressineae (e.g., *Juniperus* and *Thuya*), Taxodineae (e.g., *Sequoia*), and Podocarpineae (e.g., *Podocarpus* and *Dacrydium*), and adds another to the points of resemblance between the araucarians and podocarps.

In addition to these twigs, there was a considerable amount of wood showing the same structure. Representative sections are shown in figs. 17–19. It seems to conform exactly to the *Brachyoxylon* type, both normally and traumatically. A number of pieces had been severely wounded, and in every case one or more tangential rows of resin canals extended from the wound cap; in one specimen there were as many as four concentric series. As shown in figs. 15 and 16, the canals are often small and constricted at intervals, like those formed traumatically in the Abietineae.

Cupressinoxylon

In addition to the *Pityoxylon*, *Araucarioxylon*, and *Brachyoxylon* described above, there are others belonging to the genus *Cupressinoxylon* Kraus. They have the same combination of araucarian and abietineous tracheary pitting without bars of Sanio, but differ in having wood parenchyma scattered throughout the year's growth. The most abundant of the stems of this type is represented in figs. 20–24. The pith is large and composed entirely of parenchyma cells (figs. 20, 21), without the sclerites characteristic of the *Brachyoxyla*. The wood is composed of tracheids, rays, and wood parenchyma. The tracheids are small, with uniserial pits on the radial wall. Usually the pits are scattered (fig. 23); rarely they are crowded, but in neither case is there the slightest indication of bars of Sanio. The parenchyma cells are relatively large, being sometimes three or four times the diameter of the tracheids. The rays

are low (figs. 23, 24), with thin unpitted walls. All the parenchyma, both radial and longitudinal, whether in pith, wood, or bark, is filled with a dense black substance. The structure of the phloem is shown in fig. 22. It consists of rays, sieve tubes, parenchyma cells, and also bast fibers. These last are usually more or less crushed, but it is not difficult to see that they occur in tangential rows, alternating with rows of soft bast, just as in the case of the *Brachyoxylon* twig described above. It is noteworthy that in the former specimen the soft bast collapsed and the hard retained its natural size, while here just the reverse is true. On account of the absence of bars of Sanio, such a wood as this, though strictly speaking a *Cupressinoxylon*, cannot be affiliated with other members of that genus, e.g., *Juniperus*, *Cupressus*, *Sequoia*, etc. On the contrary, its affiliations must be with the araucarians. The type genus *Paracedroxylon* was founded by SINNOTT[5] to include wood which is *Cedroxylon* in everything but bars of Sanio; and for similar *Cupressinoxyla* the genus *Paracupressinoxylon* was established.[6] Two species were described. The first, *Paracupressinoxylon cedroides*, resembles the Cliffwood specimen in question in absence of medullary stone cells, but differs in having thick-walled, heavily pitted rays like a *Cedroxylon*. The second, *P. cupressoides*, lacks pith and bark, but has exactly the wood structure of this Cretaceous twig from Cliffwood. It seems safe, accordingly, to include both specimens as *P. cupressoides*, in spite of the difference in horizon.

The stem just described, although the most abundant of the *Cupressinoxyla*, was the only one without stone cells in the pith. Figs. 25–28 represent a more common type. In the medulla may be seen groups of sclerites, and in the wood the scattered parenchyma cells. Fig. 27 shows the low rays and well spaced pits characteristic of the mature wood of this twig. Fig. 28 shows the structure near the pith. The pits here, instead of being distant, are fairly close, and are in several places separated by bars of Sanio.

[5] SINNOTT, EDMUND, A. W., *Paracedroxylon*, a new type of araucarian wood. Rhodora **11**:165–173. *pls. 80, 81.* 1909.

[6] HOLDEN, RUTH, Jurassic coniferous woods from Yorkshire. Ann. Botany **27**:533–545. *pls. 39, 40.* 1913.

Owing to the cellulose nature of these bars, they have dropped out in the course of fossilization, and now are represented by white lines. Their occurrence near the pith of this specimen, which is clearly transitional between the Araucarineae and Abietineae, is quite in keeping with their occurrence in the cone axis of the living genus *Araucaria*, and in the first annual ring of the fossil *Araucarioxylon* described earlier in this paper. In all these cases the bars of Sanio are to be interpreted as vestiges of what was characteristic of the mature wood of their ancestors, retained only in certain conservative regions of these reduced forms. Viewed in this light, it seems evident that their presence in the first formed wood of an araucarian *Cupressinoxylon* is another indication of the derivation of the Araucarineae from the Abietineae.

This stem is representative of a considerable number which differ from each other only in slight and unimportant details. The three diagnostic features are (1) wood parenchyma scattered throughout the year's growth, (2) thin-walled ray cells, and (3) scattered pits on the radial walls of the tracheids, without bars of Sanio intervening. All these are shown in figs. 27 and 29. In such structures as arrangement and size of medullary sclerites, there is considerable divergence. For example, in the specimen represented in figs. 25 and 26, the individual stone cells are grouped in small and irregular clusters, while in the case of the twig photographed for fig. 30 they form long lines extending down the pith. There is not a little variation also in the pitting of the radial walls of the rays, though in all the horizontal and end walls are unpitted. Usually there are one to four small piceform pits to each crossfield, but in fig. 31 is shown a specimen with a single large pit. That each large pit originates from the fusion of two small pits is indicated by the occurrence of two partially fused pits, end to end. Similar stages in pit fusion were described in the case of one of the *Pityoxyla* from the same deposit. Another interesting feature of this specimen, shown in fig. 32, is the resinous exudation from the rays into the tracheids. Similar appearances are common in the living *Agathis*, and thus serve to establish another bond between these transitional Mesozoic forms and living members of the Araucarineae.

Summary

1. An *Araucarioxylon* from the Raritan Cretaceous of Cliffwood, New Jersey, shows bars of Sanio near the pith of the stem, similar to those on the cone axis of the living Araucarineae.

2. *Brachyoxyla* from the same locality are as a rule very similar to those from Kreischerville, Staten Island, differing only in such details as arrangement of medullary sclerites and structure of the bast.

3. The *Cupressinoxyla* of Cliffwood all lack cellulose bars of Sanio in the mature wood, and should on that account be placed in the new genus *Paracupressinoxylon*.

4. The occurrence of three absolutely typical *Pityoxyla*, and not a single typical *Araucarioxylon*, among these lignites seems to indicate that in tracing back the families of living conifers it is the Abietineae which remain unchanged, and the Araucarineae which become less and less like living representatives of that family. The same conclusion may be drawn from a consideration of the lignites of Staten Island.

5. The variety of structure of these Mesozoic araucarians has its bearing on the question of the monophyletic or diphyletic origin of the Coniferales. There are certain features which have been supposed to sharply differentiate the araucarians from the other families. Both fossil and comparative anatomical evidence demonstrate the fallacy of this view. As regards wood structure, every feature of the Abietineae—resin canals, bars of Sanio, thick-walled pitted rays, wood parenchyma (terminal and diffuse), even to as small and unimportant details as fusion pits in the rays and regularly alternating bands of hard and soft bast—has been found in the Araucarineae, living or extinct. As regards strobilar anatomy, EAMES[7] has shown that the stages in the reduction of the female cone are closely paralleled in various cupressineous and taxodineous genera, and the writer[8] has shown that in one Mesozoic araucarian (*Voltzia*) there was a double cone scale, like that of the living genus

[7] EAMES, ARTHUR J., The morphology of *Agathis australis*. Ann. Botany **27**: 1–38. *pls. 1–4*. 1913.

[8] HOLDEN, RUTH, Some fossil plants from eastern Canada. Ann. Botany **27**: 243–255. *pls. 22, 23*. 1913.

Cryptomeria. In view of all the facts, it seems evident that the conifers, as a whole, are derived from the same ancestral stock, and that the Abietineae are more like that stock than the Araucarineae.

This investigation was carried on in the Phanerogamic Laboratories of Harvard University, and the sincerest thanks of the writer are due to Professor E. C. JEFFREY for the lignites from Cliffwood, for the photograph of the cone of *Araucaria Bidwillii* (fig. 7), and for many helpful suggestions in the course of the work.

HARVARD UNIVERSITY

EXPLANATION OF PLATES XII–XV

FIG. 1.—*Araucarioxylon* sp.; radial section of mature wood; ×125.

FIG. 2.—Same, showing alternating and crowded pits; ×250.

FIG. 3.—Same, near the pith; toward the left, a tracheid with opposite pits and bars of Sanio; toward the right, one with uniserial pits and bars; ×500.

FIG. 4.—Same; at the left are the primary wood elements; next, a tracheid with scattered pits; and then one with opposite pits and bars of Sanio; ×250.

FIG. 5.—Same, more highly magnified; ×500.

FIG. 6.—Same, showing opposite, triseriate, and alternating pits, in every case separated by bars of Sanio; ×500.

FIG. 7.—*Araucaria Bidwillii;* radial section of cone axis in proximity to the protoxylem, showing presence of bars of Sanio; ×500.

FIG. 8.—*Pinus protoscleropitys;* radial section of mature wood showing opposite pits and bars of Sanio; ×500.

FIG. 9.—*Geinitzia Reichenbachi;* transverse section of stem, showing sclerites in pith; ×15.

FIG. 10.—Same; longitudinal section of pith; ×15.

FIG. 11.—Twig from Cliffwood; transverse section of pith, including nest of stone cells; ×15.

FIG. 12.—Same; the outer bark contains clusters of large stone cells, below which is the inner bark, consisting of alternate layers of hard and soft bast; ×40.

FIG. 13.—Same; longitudinal section of bark; ×40.

FIG. 14.—Same; longitudinal section of pith, showing nests of stone cells; ×15.

FIG. 15.—*Brachyoxylon* sp. including row of traumatic resin canals; ×40.

FIG. 16.—Same; longitudinal section; ×40.

FIG. 17.—*Brachyoxylon* sp.; transverse section of normal mature wood; ×90.

1

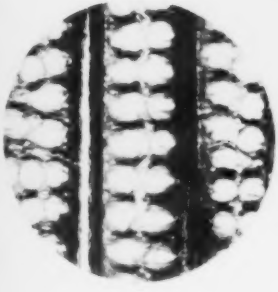
2

3

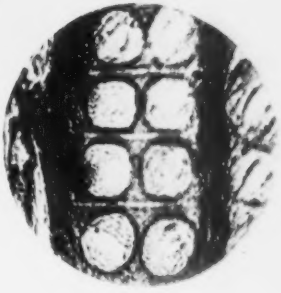
4

5

6

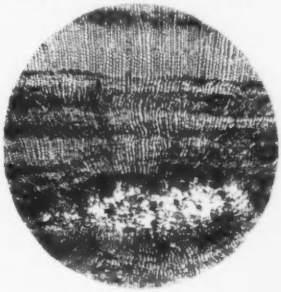


7

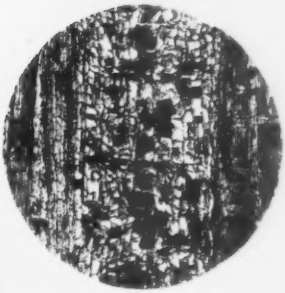


8

HOLDEN on CRETACEOUS LIGNITES

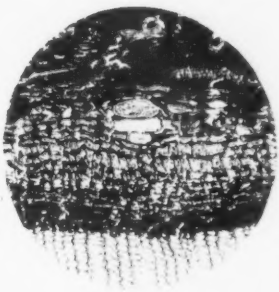


9

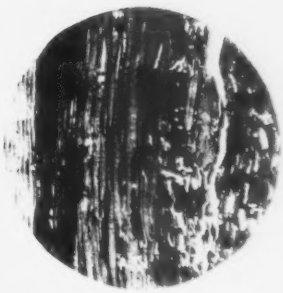


10

11

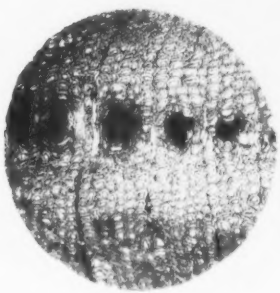


12

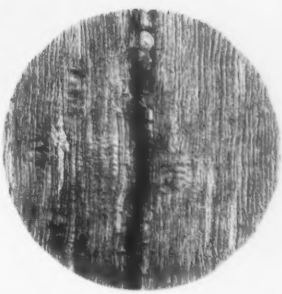


13

14

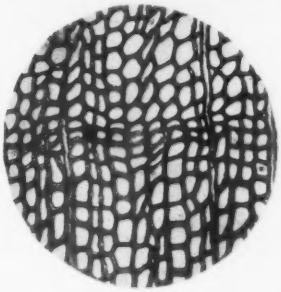


15



16

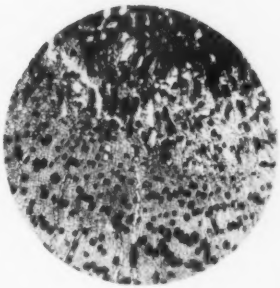
HOLDEN on CRETACEOUS LIGNITES



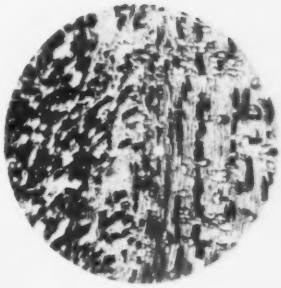
17

18

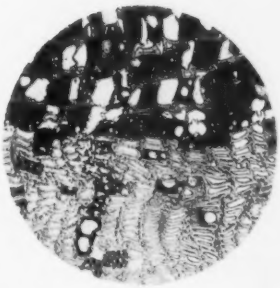
19



20

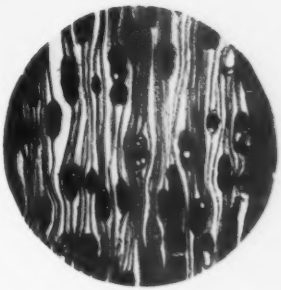


21

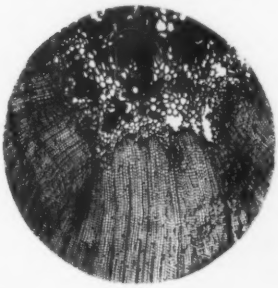


22

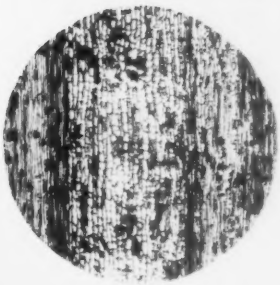
23



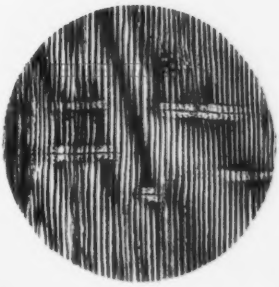
24



25

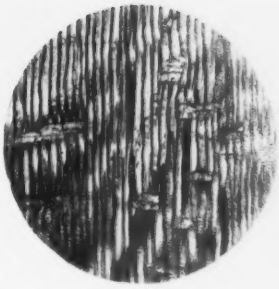


26

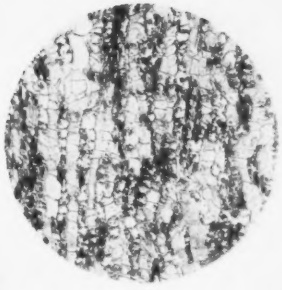


27

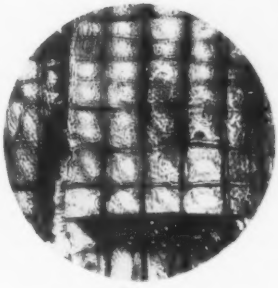
28



29



30



31

32

HOLDEN on CRETACEOUS LIGNITES

FIG. 18.—Same; radial section, showing tracheary pits sometimes crowded and sometimes scattered; thin-walled rays, with numerous small pits on longitudinal wall; ×90.

FIG. 19.—Same; tangential section, showing tangential pitting; ×90.

FIG. 20.—*Paracupressinoxylon cupressoides;* transverse section near pith, showing abundance of parenchyma cells; ×15.

FIG. 21.—Same; longitudinal section; ×15.

FIG. 22.—Same; transverse section including bark and wood; in the former the large parenchyma cells and crushed fibers may be seen; ×90.

FIG. 23.—Same; radial section, showing low, resinous rays, large parenchyma cells, and scattered tracheary pits; ×90.

FIG. 24.—Same; tangential section; ×90.

FIG. 25.—*Paracupressinoxylon* sp.; transverse section, showing stone cells in the pith, and wood parenchyma; ×15.

FIG. 26.—Same; longitudinal section; ×15.

FIG. 27.—Same; radial section of mature wood; ×40.

FIG. 28.—Same; section near pith, showing bars of Sanio; ×250.

FIG. 29.—Another specimen of *Paracupressinoxylon* showing wood parenchyma, scattered tracheary pits, and thin-walled rays; ×90.

FIG. 30.—Another specimen, showing peculiar arrangement on stone cells; ×40.

FIG. 31.—Another specimen, radial section, showing fusion pits in the rays; ×150.

FIG. 32.—Same; tangential section, indicating resinous exudations in tracheids from rays; ×90.

QUANTITATIVE CRITERIA OF ANTAGONISM

W. J. V. OSTERHOUT

(WITH FOUR FIGURES)

It is apparent from a consideration of the literature that faulty criteria of antagonism are frequently employed. It often happens that such criteria do not permit us to decide in critical cases whether antagonism exists or not.

As the study of antagonism becomes more quantitative in character it becomes necessary to have well defined standards by which antagonism may be measured. Failure to make use of such standards has led in some cases to serious misconceptions.

As an example of this the following case may be cited. Suppose a solution of a toxic salt, *A*, to be mixed with a solution of another salt, *B*, the solutions having the same molecular concentration and the same degree of toxicity. Suppose that in a mixture of 100 cc. of 0.1 M solution of *A* plus 100 cc. of 0.1 M solution of *B*, plants grow better than in either of the pure solutions. Some investigators assert that this increase of growth should not be attributed wholly to

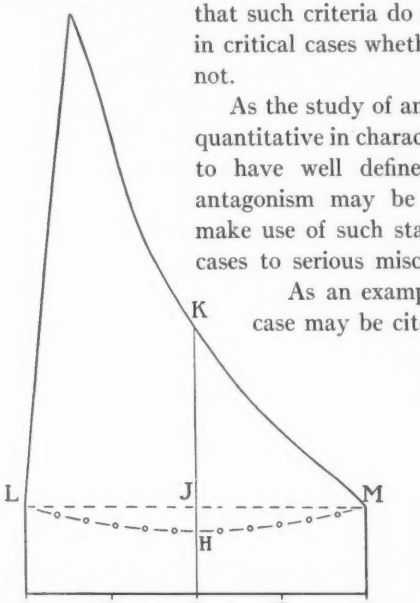


FIG. 1.—Curves showing the growth of roots in mixtures of equally toxic solutions of two salts *A* and *B:* the ordinates represent growth; the abscissas represent the composition of the mixtures, thus *A* 50, *B* 50 means a mixture in which the dissolved molecules are 50 per cent *A* and 50 per cent *B;* the horizontal dotted line (*LJM*) represents the growth which would occur if there were no antagonism (additive effect); *LKM* is the antagonism curve; *LHM*, curve expressing increased toxicity (opposite of antagonism); the quantitative expression of antagonism at the point *E* is $\frac{KJ}{JE}$.

antagonistic action, but that it is partly due to the dilution of the toxic salts, for it is evident that the concentration of each of them has been reduced from 0.1 M to 0.05 M.

It seems desirable to formulate the matter so as to obviate such misconceptions and it is clearly necessary to provide a quantitative criterion of antagonism which shall be both accurate and convenient. A graphical expression of such a criterion is seen in Fig. 1.

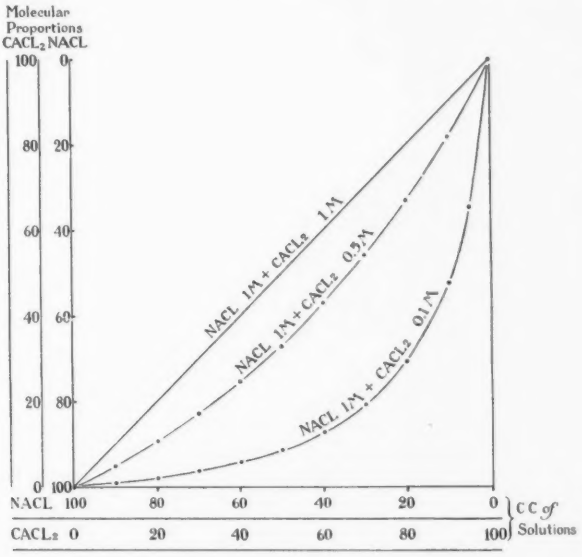


FIG. 2.—Curves showing the relation between the molecular composition of a mixture of two solutions and its composition expressed as cc. of the component solutions; see footnote 1.

In this figure the growth of the plant in a solution of the salt *A* 0.1 M is expressed by the ordinate at *C;* the growth in a solution of the salt *B* 0.1 M is expressed by the ordinate at *G;* as the ordinates are equal the solutions are equally toxic. The ordinates between *C* and *G* express the growth in various mixtures of the two solutions; thus that at *D* expresses the growth in a mixture in which 75 per cent of the dissolved molecules are *A* and 25 per cent

are *B;* the ordinate at *E* expresses growth in a mixture in which 50 per cent of the dissolved molecules are *A* and 50 per cent are *B*.[1]

It is obvious that the effect of mixing two equally toxic solutions must fall into one of the following categories.

1. The toxicity is unaltered, that is, the toxic action of the two salts is *additive*. Each salt produces its own toxic effect precisely as though the other were not present. This is expressed by the horizontal dotted line *LJM*.

It is evident that we cannot get increased growth by mixing two such solutions unless the salts have an antagonistic action. The dilution of *A* from 0.1 M to 0.05 M is exactly compensated by the introduction of molecules of *B*. Or, to put it in another way, the toxic effect depends on the number of molecules present (if both kinds of molecules are equally toxic and there is no antagonism) and it makes no difference whether the solutions are pure or mixed.

If the toxic effect depends on ions, rather than on molecules, then, since the number of ions may be somewhat increased by mixing solutions, the toxicity may be correspondingly increased; but the amount of this increase would ordinarily be negligible.

2. The toxicity is diminished, that is, the effect is *antitoxic*. We then get a curve rising somewhere above the dotted line, such as the unbroken line *LKM*.

3. The toxicity is increased. We then get a curve which somewhere falls below the dotted line, such as the line interrupted by circles *LHM*.

The considerations here set forth apply in all cases where two equally toxic solutions are mixed, whether their concentration is the

[1] In order to avoid unnecessary calculations in making up solutions with the desired molecular proportions, curves similar to those in fig. 2 serve a very useful purpose. The figures on the vertical scale denote molecular proportions, while those on the horizontal scale denote cc. of solutions. Suppose that we are mixing NaCl 1 M and $CaCl_2$ 0.5 M. If we mix 60 cc. of the NaCl with 40 cc. of the $CaCl_2$, the molecular proportions are NaCl 75 per cent+$CaCl_2$ 25 per cent. If each of the scales (vertical and horizontal) is 100 mm. long, the ordinate (measured from above downward) will in this case be 25 mm., and the abscissa (measured from right to left) 60 mm. After determining a series of such points, a curve may be drawn from which other proportions may be read off directly. Such a curve is shown in the figure (NaCl 1 M — $CaCl_2$ 0.5 M); this curve serves equally well for all solutions in which the molecular concentration of one component is twice that of the other. In the same manner the other curve NaCl 1 M — $CaCl_2$ 0.1 M applies equally well to all solutions in which the molecular concentrations of the two components are as one to ten.

same or not. Thus, if a solution of *A* 0.05 M is just as toxic as a solution of *B* 0.1 M, mixtures of the two will give a straight line (as in fig. 1) provided their effects are additive.

Emphasis should be laid upon the fact that the method of mixing two equally toxic solutions eliminates disturbances due to variations of osmotic pressure. If a molecule of *A* is twice as toxic as a molecule of *B*, a solution of *A* 0.05 M will be just as toxic as a solution of B 0.1 M, provided there are no other factors to be considered. But if the osmotic pressure of the 0.05 M solution of *A* is less than that of the 0.1 M solution of *B*, there will in many cases be better growth in the 0.05 M solution of *A*. In order to make the solution of *A* appear equally toxic with the solution of *B*, the concentration of *A* must be somewhat increased, say to 0.055 M. We thus compensate for the variation in osmotic pressure, and this compensation is not destroyed when the 0.055 M solution of *A* is mixed with the 0.1 M solution of *B*. If the effects of the salts are additive, we must therefore get a straight line, as shown in fig. 1.

It is evident that this straight line furnishes a criterion of antagonism which for quantitative purposes leaves nothing to be desired. All that is necessary is to determine what concentrations of *A* and *B* are equally toxic, mix these solutions in various proportions, and determine the amount of growth. The antagonism in any mixture may then be expressed in a very simple manner. Let the curve showing the growth in the mixtures be *LKM*. The antagonism in a mixture in which the molecules are 50 per cent *A* and 50 per cent *B* may be expressed as $\frac{KJ}{JE}$. *JE* is the growth which would have been obtained if the effect of the salts had been additive (that is, if there had been no antagonism, but each salt had produced its effect independent of the other). *KJ* is the increased growth due to antagonism; it is best expressed as percentage of *JE* or as $\frac{KJ}{JE} \times 100$.

In the same way increased toxicity (when the mixture is more toxic than either of the pure solutions) may be expressed as $\frac{JH}{JE}$. This sometimes occurs, but it is much less common than antagonism.

As an illustration of this method the results given in table I may be cited. In this case the growth in the various mixtures was in part determined directly and in part was calculated from results obtained by growing plants in mixtures having almost the same composition as the solutions given in the table.

TABLE I

MIXTURES OF EQUALLY TOXIC SOLUTIONS

Wheat (growth during 30 days) (NaCl 0.12 M + $CaCl_2$ 0.164 M)

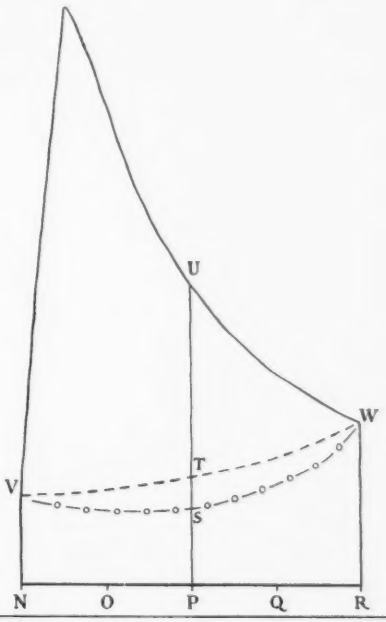
Culture solution	Aggregate length of roots per plant in mm.	Additive effect	Antagonism
$CaCl_2$	55	55	
75 per cent $CaCl_2$ 25 " NaCl	105	55	$\frac{105-55}{55}=0.91$
50 " $CaCl_2$ 50 " NaCl	180	55	$\frac{180-55}{55}=2.27$
25 " $CaCl_2$ 75 " NaCl	298	55	$\frac{298-55}{55}=4.42$
15 " $CaCl_2$ 85 " NaCl	370	55	$\frac{370-55}{55}=5.73$
5 " $CaCl_2$ 95 " NaCl	435	55	$\frac{435-55}{55}=6.91$
1 " $CaCl_2$ 99 " NaCl	300	55	$\frac{300-55}{55}=4.45$
NaCl	55	55	

The percentages refer to molecular proportions; that is, 75 per cent $CaCl_2$+25 per cent NaCl means a solution in which 75 per cent of the dissolved molecules are $CaCl_2$ and 25 per cent are NaCl.

We may now consider the effect of mixing two solutions which are not equally toxic. Suppose solution *A* 0.1 M to be twice as toxic as solution *B* 0.1 M. The effect of mixing these, if the effects were strictly additive, would be the same as mixing a solution of *A* 0.1 M with another solution of *A* just twice as toxic, or in other words would be the same as increasing the concentration of *A*. In this case the curve expressing purely additive effects would not be a straight line, but would assume the form of a curved line, convex to the horizontal axis, similar to *VSW* in fig. 3. This is evident from the curves given by MAGOWAN[2] showing growth in toxic solutions of various concentrations.

[2] BOT. GAZ. 45:45. 1908.

It would be possible to determine this additive curve experimentally, and then to express antagonism quantitatively; for example, at the point P it would be expressed as $\frac{UT}{TP}$. But the labor would be much greater than by the method of mixing equally toxic solutions. The additive curve would be determined by growing plants, not in mixtures of A with B, but in mixtures of A with another solution of A having the same toxicity as B. Or we might use mixtures of B with another solution of B having the same toxicity as A. The two methods might not give exactly the same result. This is an additional argument in favor of using equally toxic solutions.



A	100	75	50	25	0	%
B	0	25	50	75	100	%

FIG. 3.—Curves showing growth in mixtures of unequally toxic solutions: the ordinates express growth; the abscissas express the composition of the mixtures as in fig. 1; the dotted line VTW expresses the growth which would occur if there were no antagonism (additive effect); VUW, antagonism curve; VSW, curve expressing increased toxicity (opposite of antagonism); the quantitative expression of antagonism at the point P is $\frac{UT}{TP}$.

An illustration of this method is found in the results given in table II. The growth in the various mixtures (additive and antagonistic) was in part determined directly and in part was calculated from results obtained by growing plants in mixtures

having almost the same composition as the solutions given in the table mentioned.

For the sake of completeness it may be mentioned that other types of antagonism curves are found; for example, flat-topped curves and also curves with two maxima, as shown in fig. 4.

If instead of mixing two equally toxic solutions we keep the concentration of one salt constant while varying that of the other, it becomes very difficult to determine the additive curve, especially

TABLE II

MIXTURES OF UNEQUALLY TOXIC SOLUTIONS

Wheat (growth during 30 days) (NaCl 0.12 M + $CaCl_2$ 0.12 M)

Culture solution	Aggregate length of roots per plant in mm.	Additive effect	Antagonism
$CaCl_2$	85	85	
75 per cent $CaCl_2$ 25 " NaCl	125	75	$\frac{125-75}{75} = 0.67$
50 " $CaCl_2$ 50 " NaCl	195	66.5	$\frac{195-66.5}{66.5} = 1.93$
25 " $CaCl_2$ 75 " NaCl	310	60	$\frac{310-60}{60} = 4.17$
15 " $CaCl_2$ 85 " NaCl	380	58	$\frac{380-58}{58} = 5.55$
5 " $CaCl_2$ 95 " NaCl	438	56	$\frac{438-56}{56} = 6.82$
1 " $CaCl_2$ 99 " NaCl	300	55	$\frac{300-55}{55} = 4.45$
NaCl	55	55	

The percentages refer to molecular proportions; that is, 75 per cent $CaCl_2$+25 per cent NaCl means a solution in which 75 per cent of the dissolved molecules are $CaCl_2$ and 25 per cent are NaCl.

when variations in osmotic pressure influence the result. It is therefore difficult to obtain an accurate quantitative expression of antagonism by this method, and in critical cases it may be impossible to decide whether antagonism exists or not.

Emphasis should be laid on the fact that the growth of parts not in immediate contact with the solution does not furnish a trustworthy criterion of antagonism. Thus the leaves of wheat (which are not in contact with the solution) often grow well at the start in

solutions of copper salts, while the roots (which are immersed in the solution) do not grow at all. The reason is that as the solution passes through the roots to the leaves a great part of the copper is removed either by adsorption or by chemical combination.[3]

Finally, it may be noted that quantitative results are more reliable and much more easily obtained when uniform material is used. "Pure line" wheat and other seeds may now be obtained and should be used whenever possible. When they are not obtainable the following method (suggested by Professor JOHANNSEN) may be employed. Several heads of wheat are taken. One grain of wheat from each is placed in each solution (and these grains should resemble each other as much as possible). In this way each solution receives the same kinds of wheat, and an average of the growth of all the plants in any solution may be safely used for comparison with the average in any other solution. It is desirable to employ this method even when pure line seeds are used.

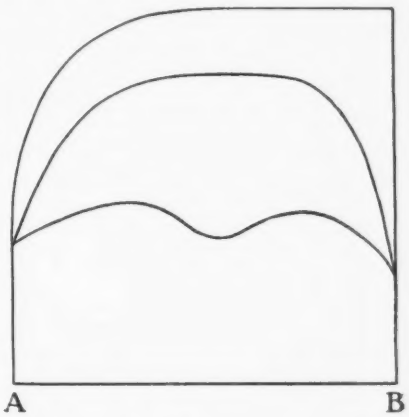


FIG. 4.—Types of antagonism curves: the ordinates express growth; the abscissas express the composition of the mixtures as in fig. 1.

Summary

The method of mixing equally toxic solutions furnishes the best criterion of antagonism, since we know at the outset just what effect each mixture must have provided there is no antagonism.

Mixtures of two equally toxic solutions must have precisely the same effect on growth as the pure solutions themselves, provided that the effects of the salts are additive. If antagonism exists there

[3] Cf. BOT. GAZ. 44:268. 1907.

is an increased growth in the mixtures. The amount of this increase, expressed as percentage of the growth obtained in the pure solutions, is the most satisfactory measure of antagonism.

The most reliable results are obtained by the use of uniform material and by taking for measurement only such parts as come into immediate contact with the solution.

HARVARD UNIVERSITY

CURRENT LITERATURE

BOOK REVIEWS

The British rusts

A volume dealing with the British Uredinales is very welcome, since it organizes in convenient form a widely scattered literature.[1] The descriptions are based upon those of SYDOW's *Monographia Uredinearum*, and all the species of which British specimens could be procured have been revised. The nomenclature of course presents unusual difficulties, but the principle of priority has been followed, subject to two conditions: (1) names given to varieties need not be adopted, and (2) names given to imperfect stages are not to be preferred, but the earliest name given to the perfect stage. Both of these conditions have been provided for by international agreements.

There are six introductory chapters dealing with general topics before the systematic presentation. The first chapter uses *Puccinia Caricis* as a typical "uredine" in giving an account of the life history of a rust. The reason given for dislodging *P. graminis* from this usual position is that its aecidium is now very rarely found in England, while that of *P. Caricis* (on nettle) is common.

The second chapter discusses the sexuality of the Uredinales and reaches the usual conclusion that there is a definite alternation of n and $2n$ generations. In the third chapter the multitudinous spore forms are discussed, with the various elisions in the life history, and the terminology arising from them.

The fourth chapter contains accounts of the life histories of certain representative Uredinales: *Puccinia graminis*, *P. Poarum*, *P. Malvacearum*, *Gymnosporangium clavariaeforme*, *Endophyllum Sempervivi*, *Cronartium ribicola*, *Melampspora pinitorqua*, and *Calyptospora Goeppertiana*. These life histories were selected to show the variations in life cycles, so far as could be done from British species.

The fifth chapter deals with specialization, including immunity; while the sixth discusses classification and phylogeny. The Uredinales are regarded as monophyletic, arising possibly from the Rhodophyceae. According to the diagrammatic scheme, the Fungi offshoot from Red Algae soon forked into two divergent branches, one ending in the Ascomycetes, and on the way giving rise to the Basidiomycetes; while the other branch resulted in Uredinales and Ustilaginales. There is also presented a scheme starting with the "primitive uredine" and resulting in the various families of the Uredinales.

The systematic part of the volume (306 pp.), with its full descriptions and numerous illustrations, makes the recognition of the British rusts as easy as

[1] GROVE, W. B., The British rust fungi (Uredinales); their biology and classification. 8vo. pp. xii+412. *figs. 290.* Cambridge University Press. 1913. 14*s.*

such a group can be. The number of species included is about 250, presented under five families.—J. M. C.

Forestry in New England

The increased attention that is being devoted to the question of forest preservation and forest restoration is being fostered and given intelligent direction by a number of publications appealing to the landowner who would make his forest-lands more valuable to himself and to the community. One of the best of these[2] deals somewhat specifically with the forestry problems of New England. Such problems appeal especially to many residents of towns and cities who own wooded land in the country, both because they often have a broader view of the economic principles involved and because intelligent forest management requires less constant attention than almost any sort of agriculture. To these and other intelligent owners of woodlands the present volume makes a direct appeal, although it may also be used with advantage by students in forestry schools.

The volume is arranged in two parts, the first dealing with such general principles of forestry as may be indicated by the chapter-headings: Silvics; Silvicultural systems; Silvicultural characteristics of New England trees; Forest planting; Forest insects and fungi; Forest fires; and Growth of trees and forests. The second part deals in some detail with the different forest regions of New England and the forest administration in the different states. A couple of maps show the distribution of the various forest regions of the New England states, while the appendix contains forest fire statistics, foresters' tables, and a bibliography.

The various topics appear to be handled with scientific accuracy, and still in a sufficiently non-technical manner to appeal to the intelligent layman; in fact, it seems to the reviewer that the mission of the book lies in such an appeal rather than in any use that may be made of it in the classroom. This would in no way lessen its usefulness, for one of the most important things in promoting the advance of forestry is the education of the general public to a conception of the problems involved, and an appreciation of the efforts that are being made for this solution. The book is well printed and attractively illustrated.—GEO. D. FULLER.

MINOR NOTICES

Rubber.—LOCK[3] has brought the subject of rubber-planting within reach of a large audience. He has combined an account of the scientific side of the subject with a certain amount of practical information that will be of service

[2] HAWLEY, R. C., and HAWES, A. F., Forestry in New England. 8vo. pp. xv+479. *figs. 140.* New York: John Wiley & Sons. 1912. $3.50.

[3] LOCK, R. H., Rubber and rubber-planting. 8vo. pp. xii+245. *figs. 22. pls. 10.* Cambridge University Press. 1913.

to the prospective planter. The science and practice of rubber-planting are both new, and important developments in both are to be expected. Some of the topics treated in the eleven chapters indicate the scope of the work: The history of the use and cultivation of rubber; The botanical sources of rubber; The physiology of latex production; Planting and harvesting operations; the pests and diseases of *Hevea;* The chemistry of India rubber.

Anyone who is familiar with LOCK's *Recent progress in the study of variation, heredity, and evolution* will know the accuracy and clearness with which the present subject is presented.—J. M. C.

The genus Sabicea.—WERNHAM[4] has published in book form a monograph of *Sabicea*, which is the first of a series of monographs on Rubiaceae. The genus belongs to tropical Africa and America, a large majority of the species being "scrambling shrubs." The number of species reaches 105, and 62 of these are described as new. This is an indication of the harvest of new species the tropics will yield when investigated. The monograph is more than a description of species, for it includes a discussion and graphic illustrations of their interrelationships.—J. M. C.

Weeds.—With the increasing demand for practical lessons for children, it is of interest to note the appearance of a booklet on weeds, by R. LLOYD PRAEGER,[5] as one of the "Cambridge Nature Study Series." The titles of the chapters suggest the contents: What weeds are; The life of a plant; On weeds in general; Seeds and their ways; The war against weeds; Some common weeds.—J. M. C.

The fresh-water flora of Germany, Austria, and Switzerland.—This very compact and well illustrated manual of the fresh-water flora of its region was planned to appear in 13 small volumes, 5 of which have appeared and have been noticed in this journal. Part 6 has now appeared,[6] dealing with three orders of the Chlorophyceae, and prepared by HEERING of Hamburg.—J. M. C.

NOTES FOR STUDENTS

Antarctic vegetation.—The activity in the south polar explorations during the past decade and a half has added somewhat to the botanical knowledge of a remarkably poor flora. In agreement with SKOTTSBERG and others, BROWN[7]

[4] WERNHAM, H. F., A monograph on the genus *Sabicea*. 8vo. pp. 82. *pls. 12*. Published by the British Museum (Natural History). 1914.

[5] PRAEGER, R. LLOYD, Weeds; simple lessons for children. 8vo. pp. x+108. *figs. 45*. Cambridge University Press. 1913. 1*s*. 6*d*. net.

[6] PASCHER, A., Die Süsswasser-Flora, Deutschlands, Österreichs, und der Schweiz. Part VI. Chlorophyceae. 3 (Ulothricales, Microsporales, Oedogoniales) by W. HEERING. pp. 250. *figs. 384*. Jena: Gustav Fischer. 1914.

[7] BROWN, R. N. R., The problems of antarctic botany. Scottish National Antarctic Expedition **3**:Bot. 3–20. Edinburgh. 1912.

accepts parallel 60° S. as a more or less natural limit for the antarctic region. Compared with the arctic flora, the antarctic is very poor, including only two species of seed plants (*Deschampsia antarctica* and *Colobanthus crassifolius*), in contrast with about 400 species in corresponding northern areas. The author finds this poverty of vegetation due not so much to the isolation of the land areas in this portion of the Southern Hemisphere as in unfavorable climatic conditions, and chiefly to the very low summer mean temperature, usually below 0° C., while in corresponding arctic regions it is well above this point. SKOTTSBERG considers that the remarkably high winds of the antarctic region must also be considered detrimental to the existence of higher plants. A second adverse factor is found in the immense number of penguins that inhabit during their nesting season every bare spot of land, thus preempting the areas with the best soil conditions for seed plants.

There are no antarctic ferns, but the mosses are represented by 52 species, of which 24 are endemic, 16 northern, and 12 southern. The fact that 16 moss species are common to antarctic and Fuegian lands is perhaps the strongest argument for the Fuegian origin of the antarctic flora. Vegetative reproduction seems the rule among these mosses, very few (6 only) being found with well developed sporophytes.

Only 6 species of hepatics have been found and but a single fungus, *Sclereotium antarcticum*, growing among *Deschampsia* on Danco Island. On the contrary, antarctic vegetation is rich in lichen display, DARBISHIRE[8] recording 106 species, of which 67 are endemic, 25 found also in New Zealand, and 32 in America, these last affording additional evidence of the probability of a migration from Fuegian lands as the origin of the antarctic flora.

SKOTTSBERG'S[9] account of the vegetation of Graham Land, including the South Shetland Islands, is quite in accord with BROWN'S. The mean summer temperature during the warmest months was −2°.14 C., although the uppermost layers of soil usually thawed for a few hours about midday. The best areas for plant life were the islands and the somewhat elevated rocks which the winds kept comparatively free from snow. Here were considerable areas of moss tundra, consisting of species of *Polytrichum*, *Pogonatum*, and *Brachythecium*, thick mats dominated by *Andreaea* and *Grimmia* upon rock surfaces, and a fairly abundant lichen flora, in which species of *Placodium*, *Lecanora*, *Buellia*, and *Neuropogon* were conspicuous. SKOTTSBERG thinks that the present flora is of post-glacial age, but that it also represents the last relic of a vegetation that was formerly somewhat more abundant.—GEO. D. FULLER.

[8] DARBISHIRE, O. V., The lichens of the Swedish Antarctic Expedition. Wiss. Ergebn. Schwed. Südpolar Exped. 1901–1903. **4**: Lief. 11. 1912.

[9] SKOTTSBERG, CARL, Einige Bemerkungen über die Vegetationsverhältniss des Graham Landes. Wiss. Ergebn. Schwed. Südpolar Exped. 1901–1903. **4**: Lief. 13. pp. 16. *pl.* 3 and *map*. 1912.

Crosses of Oenothera.—GATES[10] reports the results of various crosses between *Oenothera grandiflora* and a variant of *Oenothera rubrinervis* of DEVRIES which he has described previously under the name *Oenothera rubricalyx*. He finds *rubricalyx* pigmentation (*R*) dominant to the type of pigmentation found in *rubrinervis* (*r*), and he believes this difference to be due to a single Mendelian factor, though his proof does not seem convincing to the reviewer. Assuming this to be the case, however, the discovery that various F_1 individuals produce F_2 ratios running from 3:1 to nearly 10:1 is very interesting, particularly as the same phenomenon appeared in the segregation of tallness from dwarfness. The author says these results are "inexplicable on a Mendelian basis," and reverts to that familiar piece of biological sarcasm, the word "prepotency," as an explanation. Unquestionably there is a chance that these odd ratios may lead to a distinct genetic advance when they are finally worked out, but the word "inexplicable" is a little hasty, since it may be pointed out that some of DEVRIES' dwarf mutants (*O. nanella*) have been shown to be infected with a micrococcus.

The crucial proof that mutation is independent of hybridization, which the author feels he has obtained, came from four aberrant plants which occurred among the hybrids. Two combined characters of the DeVriesian mutant *semilata* with characters of *grandiflora*, and two combined features of *lata* with features of *rubricalyx*. One plant out of the four was examined cytologically, and its cells were found to contain 15 instead of 14 chromosomes. This furnishes definite proof, it is said, that the *lata* (or *semilata*) foliage and habit results from the presence of an extra chromosome.

A large portion of the paper is taken up with general Mendelian criticism of a very acrimonious character. It is somewhat reminiscent of the windmill tourney of a certain Spanish cavalier, for obstacles are erected, labeled "Beliefs of Mendelians," of course without any citations whatever, and tilted at with great gusto and satisfaction.—E. M. EAST.

Cruciate-flowered Oenotheras.—BARTLETT[11] has discussed certain species of the much studied genus *Oenothera*. NUTTALL named *O. cruciata* from its linear petals, and thus the character of linear or narrowly oblong petals in the genus, as contrasted with broadly obovate petals, has come to be known as "cruciateness." The origin of this character is under discussion, and BARTLETT believes that it has originated in several lines of descent. As a consequence, he regards *O. cruciata*, as it has been ordinarily interpreted, as an aggregate, which should be resolved. After determining the real type of *O. cruciata*, he

[10] GATES, R. R., Breeding experiments which show that hybridization and mutation are independent phenomena. Zeitschr. Ind. Abst. u. Vererbungslehre 11:209–279. 1914.

[11] BARTLETT, H. H., An account of the cruciate-flowered Oenotheras of the subgenus *Onagra*. Amer. Jour. Bot. 1:226–243. *figs. 2. pls. 19–21.* 1914.

adds three new cruciate species: *O. atrovirens*, *O. venosa*, and *O. stenomeres*, the first two being published in collaboration with SHULL. In connection with the segregation of these species, the cultures of the author have opened some very interesting questions that will be discussed later. For example, a new mutation was secured, which is called *O. stenopetala* mut. *lasiopetala*, and which is reserved for further discussion until its seeds have produced new plants. BARTLETT proposes a trinomial system of nomenclature, shown by the name of this mutation, for mutations of garden origin, "in order to set them clearly apart from forms of which cognizance must be taken in floras."—J. M. C.

A drought-resistant citrous fruit.—The search for hardy races of valuable plants has discovered a genus of drought-resistant citrous fruits from Australia, which SWINGLE[12] has concluded to be a new genus, to which he has given the name *Eremocitrus*. It is the Australian desert kumquat, now commonly called *Atalantia glauca* (Lindl.) Benth. It is the only member of the orange group that shows marked adaptation to desert climates, and has the general aspect of "sagebrush." It is under investigation in this country, having been sent to a number of localities for trial, so that within a year or two the limitations of its culture in the United States will be known.—J. M. C.

Pith of Osmunda.—GWYNNE-VAUGHAN[13] has described a stem of *Osmunda regalis* whose pith contains scattered tracheae. Such a "mixed pith" was described by KIDSTON and GWYNNE-VAUGHAN (1910) for the fossil *Osmundites Kolbei*. This same situation in an anomalous specimen of *Osmunda regalis* is regarded as further supporting the theory that the pith of the Osmundaceae "is phylogenetically stelar and not cortical, and that it arose by the progressive conversion of the central tracheae of a solid xylem strand into parenchyma." J. M. C.

Flora of southeastern Washington.—PIPER and BEATTIE[14] have published a manual of the vascular plants of a very interesting region, being an extension of their *Flora of the Palouse region*, published in 1901. The region covered is said to embrace the richest wheat lands of the northwest, the principal drainage systems being those of the Snake and Spokane rivers. The manual is handsomely printed, and includes descriptions of 1139 species, distributed as follows: 20 pteridophytes, 11 gymnosperms, 270 monocotyledons, and 838 dicotyledons.—J. M. C.

---

[12] SWINGLE, WALTER T., *Eremocitrus*, a new genus of hardy, drought-resistant citrous fruits from Australia. Jour. Agric. Research **2**:85–100. *figs. 7. pl. 8.* 1914.

[13] GWYNNE-VAUGNAH, D. T., On a "mixed pith" in an anomalous stem of *Osmunda regalis*. Ann. Botany **28**:351–354. *pl. 21.* 1914.

[14] PIPER, CHARLES V., and BEATTIE, R. KENT, Flora of southeastern Washington and adjacent Idaho. 8vo. pp. xi+296. Pullman: State College of Washington. 1914. Paper, $1.00; cloth, $1.20.